REFINING BRETTY WINE

Aromatic integration of Brett metabolites through microoxygenation

David Picard

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Orientador: Doutor Manuel José de Carvalho Pimenta Malfeito-Ferreira

Co-orientador: Doutora Doris Rauhut (Geisenheim University, Alemanha)

Júri:

Presidente: Doutor Jorge Manuel Rodrigues Ricardo da Silva, Professor catedrático, Instituto Superior de Agronomia, Universidade de Lisboa

Vogal: Doutora Sofia Cristina Gomes Catarino, Professora auxiliar, Instituto Superior de Agronomia, Universidade de Lisboa

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Summary

English

This work scrutinises if and how microoxygenation can affect different *Brettanomyces bruxellensis* metabolites in red wine. Through an experimental research set-up using micro-filtered tannin-rich red wine spiked with various concentrations of phenols, the work shows that microoxygenation technology adapted to small tanks helps to reduce vinyl compounds – the intermediary metabolite of the Brett metabolism – but not ethylphenol, which is chemically stable and difficult to bind. In the presence of oxygen, this reduction saw rates of up to 80% within a ten-day period. A follow-on experiment with sterile model wine further showed that the addition of oenological tannins and higher temperatures equally contribute to the reduction of vinylphenol, without producing additional ethylphenol. Aromatic integration, a concept by the American winemaker Clark Smith hence was caused only indirectly, through the reduction of vinyl compounds – and not as has been suggested by Smith through the integration of ethylphenol into the tannic structure of the wine. Building on these observations, a series of sensorial analysis panels with trained tasters from the universities of Geisenheim, Bordeaux, Lisbon and Changins demonstrated the social-cultural variability of aroma sweet spots for different types of bretty wine. While heavily phenolic characters were consistently rejected, wines with lower dosages of vinyl and ethyl compounds were considered as pleasant.

Keywords: aromatic integration, microoxygenation, Brett metabolites, phenolic character

Portuguese

Este trabalho analisa se e como a micro-oxigenação pode afetar os diferentes metabolitos da levedura *Brettanomyces bruxellensis* em vinho tinto. Através de uma pesquisa experimental adaptando a tecnologia micro-oxigenação a pequenas cubas e usando um vinho tinto previamente micro-filtrado, rico em taninos e preparado com diferentes concentrações de fenóis, este trabalho mostra que efectivamente a micro-oxigenação permite reduzir os compostos vinílicos - metabolitos intermediários do metabolismo de Brett - e não os etilfenois, que são quimicamente estáveis e difíceis de condensar. Na presença

de oxigénio, essa redução atingiu taxas máximas de 80% no período de dez dias. Uma experiência adicional com vinho modelo esterilizado, mostrou ainda que a adição de taninos enológicos e temperaturas mais elevadas (de conservação), contribuem igualmente para a redução de vinilfenol, sem produção adicional de etilfenol (em condições microbiológicas controladas, nomeadamente na ausência de células viáveis de *B. bruxellensis*). A integração aromática, um conceito do enólogo americano Clark Smith, por conseguinte, foi causada indirectamente, através da redução de compostos vinílicos - e não através da integração de etilfenol na estrutura tânica do vinho, como sugerido por Smith. Baseado nos resultados destas análises em laboratório, uma série de painéis de análise sensorial com provadores trainados das universidades de Geisenheim, Bordéus, Lisboa e Changins, manifestou a variabilidade sociocultural do 'sweet spot' para aromas fenólicos. Enquanto os vinhos fortemente fenólicos foram consistentemente rejeitados, os vinhos com doses mais baixas de compostos vinílicos e etílicos foram considerados como agradáveis.

A observação sobre a variabilidade social das preferências de gosto e caracterizações de vinho levou a uma segunda série de testes sensoriais em que as respostas diferenciadas por provadores de sexo diferente, idade e filiação institucional (Bordeaux, Vinfera Euromaster, Geisenheim, Lisboa) foram testadas umas contra as outras. Os resultados foram, eventualmente, não muito conclusivos, mostrando que os diferentes grupos amostrais independentes formam uma comunidade epistémica produzindo respostas em grande parte convergentes no que diz respeito a avaliações de aroma do vinho e preferências. As diferenças encontradas foram, principalmente, ligadas à formação, mais ou menos, consistente dos estudantes (os alunos de Bordeaux foram o grupo mais homogéneo, os alunos Vinifera apresentaram as descrições de vinhos mais heterogéneas). Além disso, obtiveram-se algumas observações inesperadas mas curiosas. Por exemplo, ao descrever os mesmos vinhos, os homens utilizaram o descritor "fruta cozida" com muito mais frequência do que as mulheres, que usaram, em alternativa, o descritor "frutos secos". Alguns descritores que fazem parte da roda de referência dos aromas usada em análise sensorial pareceu pouco compreendido por alguns painéis nacionais. Os provadores alemães pareceram incapazes de atribuir um significado claro para o descritor 'químico', e os portugueses, por seu lado, pareceram incapazes de, de uma forma consistente, utilizar o descritor 'picante'. Estas diferenças poderão estar relacionadas, respectivamente, com focos diferentes na formação institucional ou mesmo nas diferentes culturas de aroma nacional. Pode fazer sentido dar seguimento a estas observações e investigar mais aprofundadamente as culturas gustativas nacionais (ou de nível regional, étnico, classe, idade ou sexo).

Os resultados desta pesquisa têm uma série de implicações para a prática enológica. No actual nível conhecimento e know-how tecnológico, parece bastante difícil refinar vinho Bretty de uma forma controlada. A literatura académica aponta na direção de no futuro ser possível identificar linhagens nobres de *Brett*, que poderão complexificar positivamente o perfil aromático de um vinho e valorizá-lo. Poderiam ser desenvolvidos em produtos enológicos e ser inoculados antes ou depois da fermentação alcoólica, possivelmente em tanques de menores dimensões e isolados (para minimizar o risco de contaminação *Brett* não controlada), sendo mais tarde loteados com vinhos não-Brett.
Por outro lado, a adopção de uma abordagem mais "pós-moderna", como sugerido por Smith, em que leveduras *Brett*, marcadas por uma elevada biodiversidade distribuída globalmente, poderiam ser reconsideradas como parte de terroir e o processo de vinificação. Isto, obviamente, significa alterar os protocolos de vinificação e aceitar resultados inesperados - que refletem com precisão o que se entende por terroir - e também uma certa aleatoriedade, que a partir de uma perspectiva antropológica traduz, nada mais do que, a associação secular de forças divinas como parte do processo de produção e da magia do produto final: o vinho.

Palavras chave: integração aromática, microoxigenação, metabolitos da Brett, caráter fenólico

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Chapter 1. Introduction

The aim of this work was to test the pertinence of the concept of aromatic integration through micro-oxygenation, specifically as a means to manage microbial spoilages of wines resulting from infections with *Brettanomyces bruxellensis*. *B. bruxellensis* is a yeast able to metabolise different hydroxycinnamic acids (coumaric, ferulic, caffeic) naturally found in grapes into their corresponding vinyl derivatives and, in a second step, into the ethyl phenols 4-ethylphenol, 4-ethylguaiacol and 4-ethylcatechol (Chatonnet, 1993; Bisson, 2007). The presence of these molecules, together with other related compounds, especially isovaleric acids and different fatty acid esters, is responsible for aroma profiles commonly recognized as 'bretty'. These are defined by aroma descriptors ranging from the undesired 'animal', 'rancid', 'pungent', 'wet dog', 'horse sweat', and 'putrid', on the one hand, to the sometimes valorised 'earthy', 'leathery', 'smoky', and 'spicy', on the other.

The particular expression of these aromas depends on the concentrations and combinations of particular Brett metabolites that can have strong synergic effects among themselves and with the primary and secondary aromas of a given wine (Bramley, 2008). While most wine consumers and experts reject wines with a bretty aroma expression (Curtin, 2008), under certain conditions, the presence of Brett metabolites can make a wine more complex from an organoleptic perspective, by adding savoury, spicy and even animal aroma notes that certain consumers enjoy (Bisson, 2013). Brett aroma profiles can hence become, in some cases, an explicitly desired characteristic contributing to wine quality, typicity and price. Paradoxically, a certain relation seems to exist between Brett metabolite concentrations in some fine wines and the quality judgements made by renown wine critics. A recent study shows, for instance, that among a sample of French wines which received 'outstanding' rating – between 93 and 96 points – by the American wine journalist Robert Parker, the ethyl phenol concentration were between 3 and 5 times above the sensorial threshold (Knapp & Eder, 2007; Eder, 2010).

Following on from these observations, it becomes a crucial oenological question if it is possible in any way to refine the tertiary aromas caused by Brett, or by specific Brett strains, as part of the winemaking process. This question forms the overall frame of this work and is further explored in chapter 2.

As a contribution to responding to this question, this research picks up a suggestion by the American winemaker and philosopher Clark Smith. In his work on 'Integrated Brett Management', Smith (2013) claims that through microoxygenation a quantity several times the sensorial threshold of the typical *B. bruxellensis* metabolites can be 'carried' by the tannic structure of the wine and positively contribute to the overall aroma expression, complexity and quality. Taking up Smith's suggestion, this work scrutinises if and how microoxygenation can affect different *B. bruxellensis* metabolites. It focuses on two of the main families of metabolites discussed in the literature: vinyl and ethyl derivatives.

Building on Smith's work, this research tests two hypotheses:

(1) Micro-oxygenation reduces the Brett metabolites ethylphenol and ethylguaiacol by integrating them into the wine's aromatic and tannic structure (the hypothesis brought about by Clark Smith);

(2) Micro-oxygenation reduces the Brett metabolites vinylphenol and vinylguaiacol, but not ethylphenol and ethylguaiacol.

Smith's hypothesis is that that oxygen will integrate 'the aromas of varietal vegativity, oak and microbial activity […] into a good phenolic structure' (Smith, 2013: 128-9). Smith explains that oxygen will refine the colloids in such a structure and make more surface area available for aromatic integration. The presumed chemical pathway underlying this hypothesis is that oxygen will react with the ethanol in the wine and produce acetaldehyde, which in turn binds tannins, anthocyanins and the phenols metabolised by the Brett yeast, forming larger polymers – and also pyrano structures as suggested by Morata et al. (2006, 2007; cf. also Marquez et al. 2013). However, in his suggestions, Smith seems to oversee that ethyl derivatives in particular are neutral molecules that are difficult to bind into larger structures. This seems to be why no real fining agents for bretty wines have been found so far (Suárez et al., 2007). Also, to our knowledge it has not been demonstrated anywhere that ethylphenols can be bound, in any way, into the tannic structure of a wine.

Actually, Morata's work on the formation of vinylphenolic pyranoanthocyanins is focused on vinyl compounds, which are the primary Brett metabolites and ethyl phenol precursors. Morata demonstrates that reducing the availability of vinyl compounds through the formation of stable pigments will indirectly impair the formation of secondary ethylphenolic Brett metabolites. Subsequently, building on Morata's findings, the second, alternative hypothesis is that oxygen facilitates chemical reactions between different flavonoids – anthocyanins and possibly also tannins – and the non-neutral molecules of different vinyl derivatives. In this

sense ‘aromatic integration’ mainly takes place after the first step of the twofold *B. bruxellensis* metabolism of hydroxycinnamic acid, reducing and transforming the main vinyl compounds: 4-vinylphenol, 4-vinylguaiacol and 4-vinylcatechol and, by that means, decreasing their availability for further metabolic conversion into ethyl phenols.

To test the two hypotheses, an experimental microoxygenation setup was built, using 2013 German red wine spiked with different quantities of ethyl and vinyl phenol in 25L tanks, and the application of around 30 mg/L of effectively taken up oxygen per month over the duration of 10 days. The experiment was run in March and April 2015 in the cellar of the oenology department of the University of Geisenheim in Germany. To prevent yeast activity during the experiment, the wines were micro filtered at 1,5 µm. The experiment included one full repetition. Before and after the experiment, all wines were tested for their respective phenolic levels, by means of gas chromatography and photo spectrography, so as to be able to measure changes in the respective phenolic concentrations and intensity and hue shifts in the pigment structure (indicative for the stabilisation of pigments and the formation of pyranoanthocyanins). Moreover, a series of taste panels with triangular, ranking and descriptive tests was organised to verify if organoleptic changes could be observed between wines with and without microoxygenation, and to explore how different tasters rank and describe the respective wines. A lab-based post-experiment trial of vinyl compound reductions in the presence of oxygen and oenological tannins was carried out in model wine, to reconfirm the main results of the experiment in a better-controlled environment. A detailed description of the methodology and research set-up is developed in chapter 3. The results are presented and discussed in chapters 4 and 5.

The project was supported by an (unpaid) ERASMUS+ Internship fellowship by the University of Lisbon. It was hosted at the microbiology and oenology departments of the University of Geisenheim, under the supervision of Doris Rauhut (microbiology) and Maximiliam Freund (oenology). The time-plan and tasks of the project are shown in Table 1.

Table 1: Research project calendar

Feb-Mar 2015	• Preparation and analysis of base wine • Development of small tank microoxygenation set-up • Sensorial analysis panels • Preparation of tanks, spiking with phenols • Microbial and chemical analysis of all wines
Mar-Apr 2015	• 10 days of microoxygenation
Apr-May 2015	• Triangular and preference tests with expert taste panel • Preference and descriptive tests with expert panels
May-Jun 2015	• Data analysis, writing up.

Chapter 2. Reviewing *B. bruxellensis* and the paradox of 'bretty' wine

The aim of this chapter is to review the academic literature investigating what Brett is, how its metabolisms function, how it can be controlled and how its strain diversity can differentially influence the quality and value of wine. Through this review, the chapter also attempts to shed light on the commercial and sociocultural paradox of bretty wines, which more than any other aroma family polarizes the wine-drinking humanity.

2.1 Exploring the Brett paradox

Since the 1990s, the 'brettyness' of wine has been the object of a sometimes-heated debate among global winemaking scholars and experts. One central issue at stake here was if, or if not bretty wine aromas – also called 'phenolic' aromas – could be linked to terroir. A turning point in this debate was reached in 1998, when the American wine collector, Charles Collins sent samples of two widely renowned vintages of Château de Beaucastel from the Châteauneuf du Pape region in France to a lab, for analysis. He wanted to know if the wine's typical aroma profile – defined by its earthy, leathery and animal characteristics – was linked to terroir, as its producers and parts of the wine community claimed it, or if it simply was infected by *B. bruxellensis*. The lab found small amounts of dead Brett cells in all bottles, plus very significant amounts of the Brett metabolite 4-ethylphenol (hereafter: 4EP): 897 µg/L in the 1989, and 3330 µg/L in the 1990 vintage. It seemed that the wines were 'bretty' because of a systematic microbial spoilage occurring in the winery's cellar, and not, as it had been claimed, because of the specificity of its terroir, soil or climate.

And now the paradox. During the early 2000s, the 1989 and 1990 vintages of the Beaucastel consistently received top reviews by international wine experts. In 2003, Jancis Robinson described the aroma profile of the 1990 Beaucastel – the one with more than 3330 µg/L of EP in the sampled bottle – in terms of 'herbs and leather and undergrowth but still fragrant on the mid palate; silky and sweet in the mouth with a touch of black truffle and tobacco leaf' (Robinson, 2003, no page numbers). She gave the wine an excellent rating of 18 out of 20. In the same vein, other wine reviews positively emphasized the 'earthy leather notes and cherries pushing through on the mid-palate' (Yorkshire Post, 2012) or the 'wonderfully

perfumed open nose [that] shows exotic spices and some animal notes with medicinal overtones' (Wine Anorak, 2015, no page number). In a 2004 review, the online wine magazine WineAnorak.com (2004) concluded that 'it's bretty but it works' and attributed the wine a first-rate score of 95 points out of 100. In 2014, the 1990 Beaucastel was traded for a staggering EUR 142.00 at the online wine shop Comptoir des Millesimes (2014).

The case of the Beaucastel vintages is by no means special. In a pioneering work published already in 1992, Chatonnet found that among a sample of 100 high value French wines, more than 30% showed significant traces of Brett infections. He stressed that some wineries were almost systematically affected, while others not, even within the same region. (He worked with Bordeaux wines). Moreover, he observed that most wines – both white and red – were free or contained only very small amounts of ethylphenol at the end of the alcoholic and malolactic fermentations, and that the phenolic levels mostly only increased during ageing in oak barrels, especially when using second hand barrels and during the warmer summer months. Chatonnet suggested that viable Brett cells present in the barrels caused the phenolic character. A lab-based experiment comparing a control sample with a sterile filtered sample of red wine inoculated with *B. bruxellensis* confirmed his initial hypothesis. After thirty days at 25 degree Celsius, the inoculated sample had developed a very significant 'bretty' (or 'phenolic') character. Chatonnet had proven that the level of ethylphenol in the wine, responsible for the bretty character developed during barrel aging or in a contaminated bottle, was effectively related to the presence of populations of *B. bruxellensis* – and not, as previously assumed, to malolactic fermentation (Dubois, 1983).

Taken from another perspective and in a way reinventing the notion of terroir, it could be argued that each winery's specific microbial flora and fauna is actually part of its specific typicity-bearing terroir. Recent studies on the particularity of endemic Brett strains found in specific vineyards and cellars seem to corrugate such an argument. In a comparative work in Bordeaux, Renouf et al. (2006) demonstrate the terroir characteristic of what they call specific 'microbial consortiums' found in one winery, but not in others only a few kilometres away. These winery-specific microbial consortiums included specific strains of *B. bruxellensis* which contributed to the typicity of the produced wine. Similar studies on the geographical typicity of specific endemic Brett strains in other wine regions seem to confirm these initial findings (Conterno et al., 2006; Roeder et al., 2007; Curtin et al., 2007; Agnolucci et al., 2009; Puig et al., 2011).

In this sense, one Brett strain does not equal another and Brett can, under certain conditions, be a value-adding part of a winery's product portfolio and brand identity. In a

systematic survey of French wines, the Austrian wine scientist Eder brings the underlying paradox to the point: those wines that the American wine critic Robert Parker attributed more than 93 points almost systematically contained high amounts of Brett metabolites (Eder, 2010). He concludes that, on the one hand, Brett is a main cause for microbial flaws observed in wines worldwide, on the other, it can make wine more valuable and contribute to its complexity and typicity.

2.2 Brett metabolism, ecology and biodiversity

As it was already mentioned in the Introduction chapter above, *B. bruxellensis* is a yeast able to metabolise the hydroxycinnamic acids (coumaric, ferulic, caffeic) naturally found in grapes into their corresponding vinyl derivatives (4-vinylphenol, 4-vinylguaiacol and 4-vinylcatechol) and, in a second step, into the ethyl phenols 4-ethylphenol, 4-ethylguaiacol and 4-ethylcatechol (Heresztyn, 1986; Chattonnet, 1993; Bisson, 2007). The first step consists here in an enzymatic decarboxylation of hydroxycinnamic acids leading to the production of hydrostyrenes (cf. Fig 1). This enzymatic reaction can also be provoked by other microbial agents, namely *Saccharomyces cerevisiae* and *Lactobacillus plantarum* (Rodriguez et al., 2008; McKenna, 2014). The second step in the production of ethyphenol, however, is largely dominated by the yeast *B. bruxellensis* and its ability to effect an enzymatic vinyl phenol reduction.

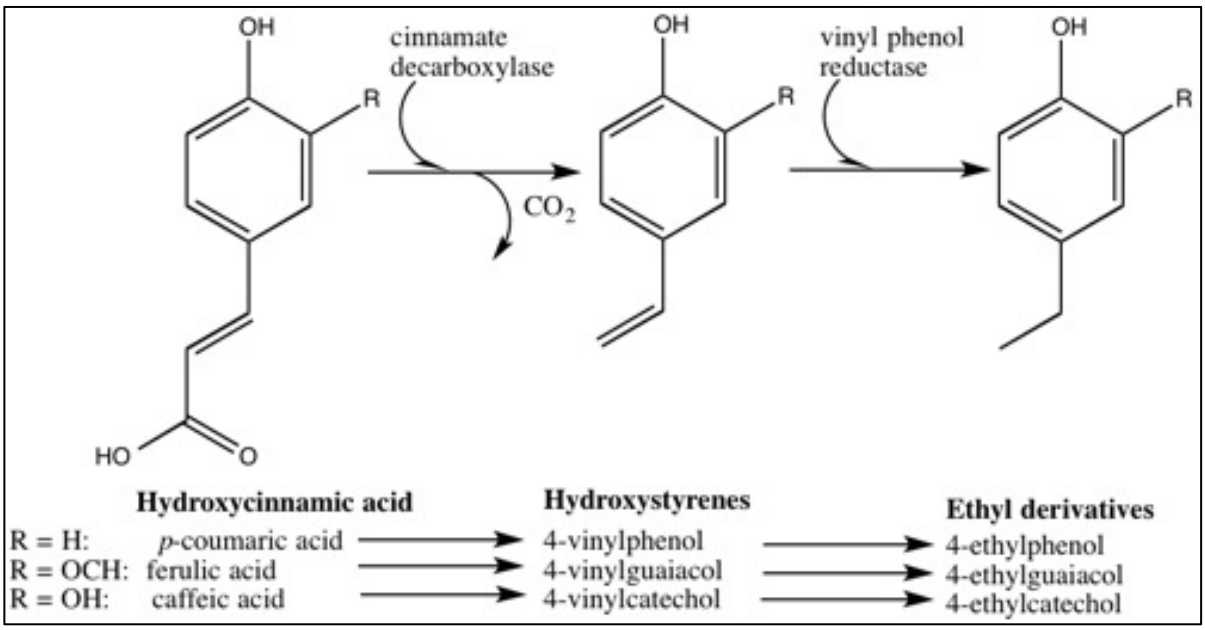

Fig. 1: Enzymatic transformation of hydroxycinnamic acid by *B. bruxellensis*

B. bruxellensis is a natural yeast, first reported in cider in 1912 and in wine in 1952 (cf. Chatonnet 1992; Suárez et al, 2007; Bisson et al., 2007; Wedral et al., 2010). It is extremely

versatile and resistant, and seems to easily adapt to new environmental conditions. It needs less than 1mg/l sugar to survive. *B. bruxellensis* is sensitive to ethanol, yet most strains are tolerant to 10% volume of ethanol, and thrive at pH 2. *B. bruxellensis* grows in both aerobic and anaerobic conditions (Ciani et al., 2003), and many strains resist to 30mg of free SO_2/L (Du Toit et al., 2005). It can use different types of sugar to survive, can produce acetic acid, although this is strain-dependent. *B. bruxellensis* uses nitrogen to grow variably relying on ammonium, proline, and arginine as a nitrogen source (Conterno et al., 2006). Some strains produce β-glycosidase that can break up the bond between volatile aromas and sugar to use the glucose (Mansfield et al., 2002). The yeast easily outperforms all non-*Saccharomyces* yeast species once alcoholic fermentation is over. The highest risk for Brett metabolic activity therefore is observed after the alcoholic fermentation: during malolactic fermentation, barrel ageing, and in the bottle.

Brett cells are found all along the wine production chain. They are observed in the vineyard and in the winery, especially on equipment that is difficult to sterilize, like vats and pumps. Chatonnet et al. (1990) argue that the greatest risk for Brett infection in the cellar occurs when using second hand barrels in which the yeast easily survives even when rigorous sanitation measures are applied (cf. Malfeito-Ferreira, 2011 for a review of such measures). Ultrasonic technology exposing entire barrels to ultrasonic waves currently seems one of the most viable solutions available (Yap & Bagnall, 2009). Nevertheless, Chatonnet et al. (1990) show that both new and second hand barrels can play host to Brett cells. The Bordeaux winemakers with whom Chatonnet and his team collaborated explained that, from their experience, in new barrels the 'phenolic' character would usually appear after 9 months. Practically, from the current state of knowledge, this time period is needed to infect the barrels with the yeast and allow it to grow to a critical population. The infection of such new barrels has been attributed among others to the practice of topping up, whereby the yeast is carried into hitherto uncontaminated barrels, both through cellar equipment and the actual wine used to top up (Loureiro & Malfeito-Ferreira, 2003; Renouf et al., 2007; Roeder, 2006). At the same time, under certain circumstances, the yeast may already be in the new barrels when these are delivered. In a comparative study in Australia Spillmann (2000) investigated Brett infections in relation to barrel fabrication techniques and processes. He observed that the eventual Brett risk significantly differed according to the type of place in which the material used for the barrel production was seasoned. In some places Brett cells were likely to be found in the natural environment and able to infect the wood later used to build the barrels.

The yeast can as well survive in small numbers all through the winemaking process, carried into the cellar from the original vineyard and grow in population once the conditions are right (Conterno et al., 2006; Barata et. al, 2012).

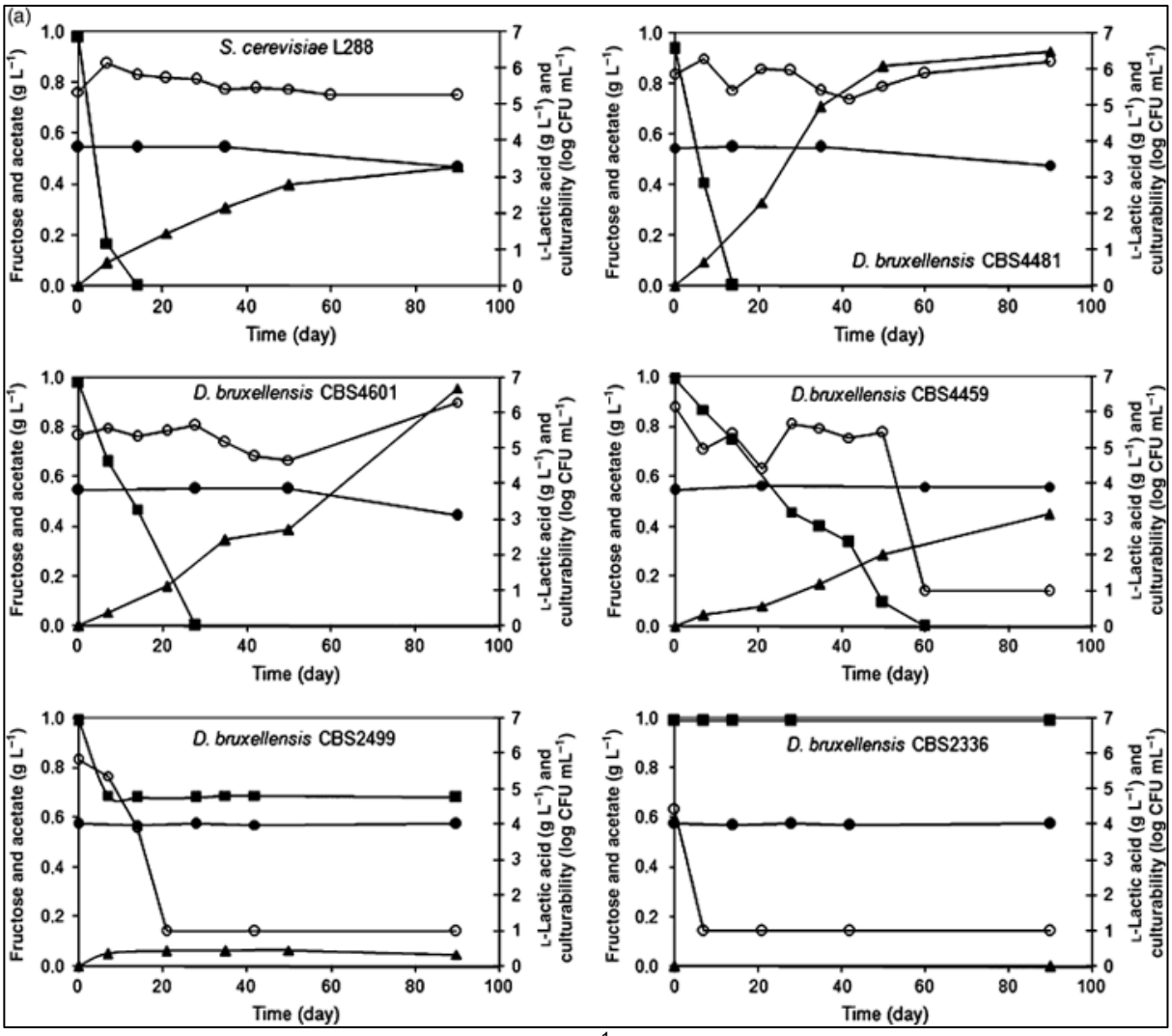

Fig. 2: Growth comparison among five strains[1] of *B. bruxellensis* and *S. cerevisiae* L288 (Vigentini et al., 2008)

[1] *B. bruxellensis* strains from CBS collection in the EWOA (a) and in the EWA (b). Fructose (▪), acetate (▲) and l-lactic acid (•) concentrations are expressed in g L^{-1}. Culturability (○) is reported as log CFU mL^{-1}.

[2] In the course of his graduate studies at the University of Geisenheim, the author has

A significant number of genetically distinct natural strains of Brett from different geographical areas have been identified, with sometimes radically different metabolisms (Conterno et al., 2006; Curtin et al., 2007; Roeder et al., 2007; Joseph et al., 2013). In a comparative study of the growth of different *B. bruxellensis* strains in synthetic wine, Vigentini et al. (2008) showed that each strain has a distinct metabolism leading to very different levels of volatile phenol production in the respective test wines. This production largely depended on the differentiated abilities by the strains to use fructose, acetate and lactic acid for their growth (cf. Fig. 2). Those with a higher metabolic ability grew faster and to larger populations, and subsequently also produced more ethyl derivatives (and 'brettyness').

Observing this genetic and bio-functional diversity of Brett strains, the challenge for microbiological and oenological research is to identify those strains that produce Brett aromas considered as pleasant, while finding means to control the others (Bisson, 2013). Moreover, if geographically specific strains produce biochemically specific Brett aromas recognized as terroir-typical, further research is needed on such potentially noble endemic Brett strains and their appearance in specific vineyards or cellars. The actual threat to such endemic strains is the global trade in second hand winemaking material, bearing the risk of microbial contaminations and colonisations by exogenous strains superseding or even eliminating the endemic strains. To manage this risk, it may be a good policy recommendation at OIV level to prohibit trade in second hand barrels altogether or only allow such trade when barrels are treated with ultrasound sterilisation technology; the only technology currently available that seems to work with 100% efficiency (Yap & Bagnali, 2009. Cf. Section on 'Controlling *B. bruxellensis*' further below in this chapter).

2.3 Organoleptic properties of Brett metabolites

The 'brettyness' of wine is mainly about aroma, although impacts upon texture and mouth-feel were equally observed (Bisson, 2013). The sensory detection thresholds of different Brett metabolites vary widely as a function of the type and style of wine, and the grape varieties used (Fugelsang & Zoecklein, 2003; Botha 2010): between 120 to 1200 µg /L for 4-ethylphenol, 70 to 150 µg/L for 4-ethylguaiacol (Chatonnet et al., 1993; Curtin et al., 2008; Eder, 2010). Wines rich in tannins or green aromas are able to mask Brett aromas and hence have significantly higher detection thresholds. In an experiment run by the Australian Wine Research Institute, the 4-ethylphenol thresholds in a green and in a heavily oaked Cabernet Sauvignon wine respectively increased to 425 µg/L and 569 µg/L (Curtin et al., 2008). Similar observations including on the widely unpredictable range of detection thresholds were made by Eder (2010) in Austria and Botha (2010) in South Africa. The

presence of 4-ethylphenol and 4-ethylguaiacol has a cumulative effect: if the medium sensory detection threshold for 4-ethylphenol alone is around 600 µg/L, a mixture with 4-ethylguaiacol brings such a threshold down to around 400 µg/L (cf. Curtin et al., 2008; Eder, 2010). Brett can sometimes only appear in the process of aging, not as a result of microbiological spoilage but simply because as wine ages, primary fruit aromas reduce which can reveal Brett characters that have earlier been masked.

As it was explained in the opening lines of this chapter, the bretty – or 'phenolic' – character of wine can variably be perceived as pleasant or unpleasant. In the academic literature, different aroma descriptors are – not always consistently – associated with different metabolites (Chatonnet et al., 1993; Ferreira et al., 2000; Bisson et al., 2007; Eder, 2010; Botha 2010), as it is shown in Table 2. Eder (2010) argues that increasing concentrations of the chemical compounds usually lead a progressive increase of a specific aroma note; an observation that our research will contest (cf. Chapter 4. Results).

Table 2: Brett metabolites and corresponding aroma descriptors

	Bisson et al., 2007 based on Chatonnet et al., 1993; Ferreira et al., 2000	Eder, 2010
4-ethylphenol	Plastic, Band-Aid	Leather, sweaty, animal
4-ethylguaiacol	Smokey, clove	Clove, cinnamon
4-ethylcatechol	Horsy	Chemical, medical
4-vinylphenol	*Not reported*	Medical, spicy candy
4-vinylguaiacol	*Not reported*	Medical, bacon, rubber, hay, barnyard
Isovaleric acid	Rancid, barnyard	Vegetable, fruity, lactic
Tetrahydropyridines; 2-Acetyl-1-pyrroline	Mousy	*Not reported*
Ethyl-2-methyl butarate	Fruity	*Not reported*
Combination of 4-ethylphenol, 4-ethylguaiacol & IVS (1:3,4:20,8)	*Not reported*	Sweaty-smoky, leather-smoky, manure-smoky

In these previous academic works, tasters often considered smoky and spicy aromas as pleasant while sweaty, putrid and chemical aromas as unpleasant. In many cases, the leathery, animal and medical-chemical aromas of bretty wines frequently polarized the taste panels into two groups, one clearly judging these as pleasant and the other clearly disliking them (cf. Blake-Grey, 2015).

Moreover, tasters from different cultural backgrounds often have quite radically different olfactory memories and references used to define an aroma. For instance, as reported from

a trial at the University of California at Davis (Blake-Grey, 2015), many people brought up in the US associate the smell of 4-ethylphenol with the antiseptic previously used in Band-Aid and reject it as a wine fault. To the contrary, many Chinese tasters seem to lack this reference to Band-Aid and judge 4-ethylphenol positively, by associating it to Chinese 5-spice.

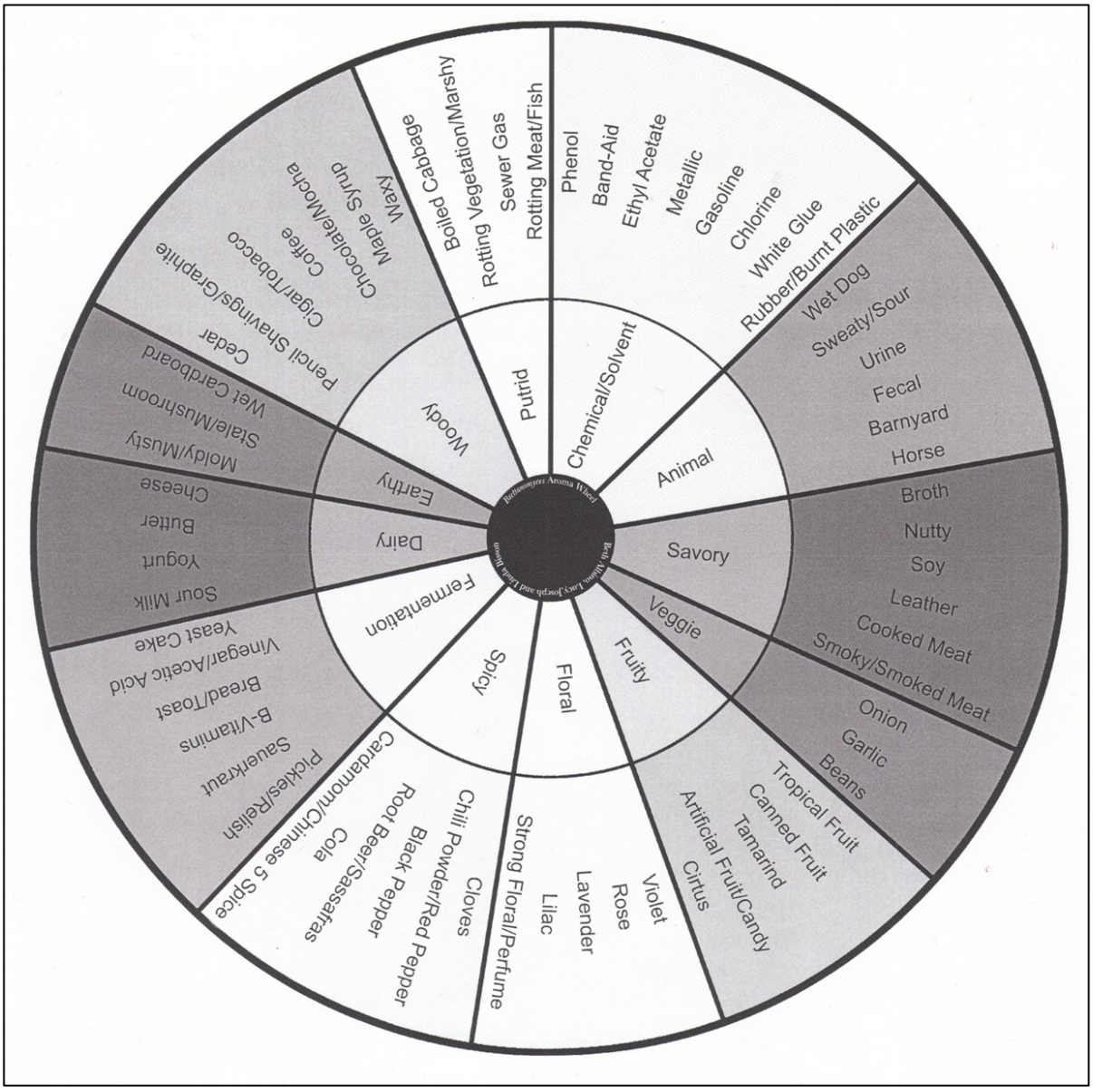

Fig. 3: Brett aroma wheel developed by Bisson & Joseph (Adams, 2013)

The cultural background of wine consumers and their – socially framed – repertoires of sensory references and related aesthetic judgements seems crucial for the wider understanding of wine appreciation. Wine consume and appreciation hence is subjected to different normative frameworks prescribing, in a nutshell, how a wine is expected to taste

and, correlated, its commercial value. In this sense, all types of wine consumers (including wine experts) are socialised to specific wine drinking cultures which, in turns, are subjected to changing fashions, knowledge and aesthetic doctrines. With regard to 'bretty' wines two broadly opposed views can be observed. One, defending a pure-fruit-aroma-driven approach to winemaking, considers Brett as a fruit-aroma-masking spoilage that needs to be controlled and, at best, eradicated. Until today, this view has been widely dominant among many oenologists, sommeliers, wine academics and wine journalists (Curtin, 2015). The other view, which has gained in momentum over the past years, suggests instead considering Brett as a particular family of fermentative aromas. Bisson and Joseph, from the University of California at Davis went so far as to suggest a new aroma wheel including various Brett aroma notes (cf. Fig. 3).

2.4 Controlling Brett

The control of Brett represents one of the currently most challenging issues in winemaking technology. The challenge is amplified by the observance of progressively hotter climates leading to higher sugar levels and pH in the musts, which increases the risk of Brett cell growth. This is further amplified by current consumer demands for higher levels of residual sugar in red wines and at the same time for lower SO_2 levels. Measures to control Brett populations during the winemaking process include hygienic, oenological and viticultural practices.

Such measures start in the vineyard where the choice of planted varieties, canopy management, adobe addition, water management and harvest date in a given climate variably impact on sugar levels, phenol precursors and yeast available nitrogen (YAN) in the must (Tomasino, 2006). Some varieties – for example Mourvèdre – are richer in 4-ethylphenol precursors and hence need more careful handling at the cellar if bretty aromas are to be avoided. Excessive fertilizer addition, especially of nitrogen may later lead to excessive YAN values in the must that are eventually not fully used by *S. cerevisiae*, hence eventually supplying nutrients for the growth of *B. bruxellensis* cells after the end of the alcoholic fermentation (Childs et al, 2015). The same logic applies for nitrogen applications directly into the must before and during fermentation (Simos et al., 2007). For instance, the widespread oenological practice of adding diammonium phosphate (DAP) to boost the growth of yeast during fermentation can inhibit the breaking down of naturally available amino acids, which can then become a source of nutrients for Brett (Conterno et al., 2006; Childs et al., 2015). As a solution, the levels of YAN in the fermenting must should be

adjusted to the distinctive nitrogen uptakes of specific commercial and natural yeasts (Barrajón-Simancas et al., 2011).

Measures to control Brett in the cellar are based on good general hygiene and the use of best available sterilisation practices. However, technologies proven to be close to 100% efficient do only emerge. Hitherto, the anti-microbial treatment of material and equipment was done mostly by chemical sterilisation (using chloral-, sulphur-, or ozone-based inactivating agents) and thermal inactivation. Both types of technology prove partly insufficient as Brett cells manage to survive in inaccessible places in the cellar, inside the tubing and in the small cracks of wooden barrels (Zuehlke et al., 2013). A new, seemingly more efficient approach is based on the physical inactivation of Brett cells, using electromagnetic wave technologies, pulsed electric fields (PEF) (Couto et al., 2013) and high power ultrasonics (Yap et al., 2009).

Table 3: Minimal inhibitory concentrations and minimal biocide concentrations for different antimicrobial agents (adapted from Portugal et al., 2014)

Antimicrobial agent	Range	Microorganism	MIC		MBC	
			MIC_{50}	MIC_{90}	MBC_{50}	MBC_{90}
Ethanol	8–20 %	*S. cerevisiae*	>20 %	>20 %	>20 %	>20 %
		B. bruxellensis	>20 %	>20 %	>20 %	>20 %
PMB	6.25–0.003 g/L	*S. cerevisiae*	780 mg/L	780 mg/L	780 mg/L	780 mg/L
		B. bruxellensis	48 mg/L	96 mg/L	96 mg/L	96 mg/L
PMB + ethanol[a]	6.25–0.003 g/L	*S. cerevisiae*	48 mg/L	390 mg/L	190 mg/L	390 mg/L
		B. bruxellensis	24 mg/L	48 mg/L	48 mg/L	48 mg/L
Tannins	4–0.002 mL/L	*S. cerevisiae*	>4 mL/L	>4 mL/L	>4 mL/L	>4 mL/L
		B. bruxellensis	0.5 mL/L	1 mL/L	>4 mL/L	>4 mL/L
Tannins + ethanol[a]	4–0.002 mL/L	*S. cerevisiae*	0.5 mL/L	0.5 mL/L	4 mL/L	>4 mL/L
		B. bruxellensis	0.125 mL/L	0.125 mL/L	0.25 mL/L	>4 mL/L
Chitosan	250–0.12 mg/L	*S. cerevisiae*	>250 mg/L	>250 mg/L	>250 mg/L	>250 mg/L
		B. bruxellensis	62 mg/L	62 mg/L	250 mg/L	>250 mg/L
Chitosan + ethanol[a]	250–0.12 mg/L	*S. cerevisiae*	62 mg/L	125 mg/L	250 mg/L	>250 mg/L
		B. bruxellensis	31 mg/L	62 mg/L	62 mg/L	>250 mg/L
DMDC	5–0.002 g/L	*S. cerevisiae*	156 mg/L	312 mg/L	625 mg/L	1250 mg/L
		B. bruxellensis	78 mg/L	156 mg/L	312 mg/L	625 mg/L
DMDC + ethanol[a]	5–0.002 g/L	*S. cerevisiae*	5 mg/L	30 mg/L	625 mg/L	1250 mg/L
		B. bruxellensis	10 mg/L	60 mg/L	120 mg/L	625 mg/L

MIC minimum inhibitory concentration, *MBC* minimum biocide concentration

As part of winemaking operations, existing Brett cells can also be directly eliminated from the wine or their growth inhibited. The longest established and hitherto most commonly used antimicrobial agent is sulfur dioxide (SO_2), which at levels above 30mg/L proves relatively efficient to control Brett cell growth (Umiker, 2007). Yet, in a context in which wine consumers (and journalists) sometimes identify the presence of SO_2 in wine as an allergenic

agent and potential public health risk, alternative microbial inhibitors have emerged and been approved over the past 10 years. In a comparative study, Portugal et al. (2014) verify the good antimicrobial effect of some of the most widely used new products: potassium metabisulfite (PMB), oenological tannins and chitosan in the presence of ethanol, and dimethyl dicarbonate (DMDC) in the absence of ethanol. Their study (cf. Table 3) shows that PMB has the highest efficacy in concentrations within the currently permitted limits (Portugal et al., 2014: 641).

Another approach championed by Morata et al. (2013) is to reduce the available precursors of ethylphenol in the wine: vinylphenol (the first product of the Brett metabolism) and hydroxycinnamic acids as vinylphenol precursor. In their earlier work, Morata et al. (2006, 2007) show that during fermentation vinylphenol can spontaneously condense with grape anthocyanins producing chemically stable vinylphenolic pyranoanthocyanins. The available vinylphenol is hence reduced (while simultaneously the pigments stabilized), and even if viable Brett cells are present, the amount of produced ethylphenol is significantly reduced. In an ideal situation, the wine will never get to a stage at which it smells bretty.

Morata et al. (2013) point at another concurrent crucial issue. Vinylphenol is produced by yeast hydroxycinnamate decarboxylase of hydroxycinnamic acids. The total available hydroxycinnamic acids are made both from free hydroxycinnamic acids present in the grape and from tartaric esters of hydroxycinnamic acids (TE-HCA). Morata et al. (2013) show that the latter compounds are being in higher amounts than the free hydroxycinnamic acids, and that they constitute a kind of reservoir that persists at the end of fermentation, and that can slowly release hydroxycinnamic acids during barrel ageing. Brettanomyces can metabolise these progressively supplied free hydroxycinnamic acids first into vinylphenol and then into ethylphenol; the wine will become bretty. Morata et al.'s suggestion is to use cinnamyl esterases as an oenological application already early on in the winemaking process (during fermentation) in order to hydrolyze the tartaric esters of hydroxycinnamic acids and release the previously bound hydroxycinnamic acids. Through the addition of hydroxycinnamate decarboxylase yeasts, these hydroxycinnamic acids can then be enzymatically transformed into vinylphenol which in turns can bind to anthocyanins, leading to the chemical formation of the above-mentioned chemically stable vinylphenolic pyranoanthocyanins.

An additional or alternative microbial control technology is micro- or sterile filtration. Viable Brett cells have a diameter of approximately 5-7 µm and their filtration at 1,5-3 µm should therefore allow a winemaker to eliminate all viable cells (Umiker et al., 2013). This is of course not practical when the presence of autolysed (dead) yeast cells are required as a

source for mannoproteins adding texture and body to the wine, for instance during barrel aging, to bind tannins from new oak barrels and interact with flavour compounds (Caridi, 2006). However, it is definitely an option at the bottling stage, to avoid the presence of viable Brett and other yeast cells in the bottle.

2.5 Aromatic integration of Brett metabolites through microoxygenation?

Some of the before-mentioned oenological-practice based approaches to controlling Brett have been summarized in a radically alternative approach that the American winemaker Smith (2013) defines as 'Integrated Brett Management'. He suggests three key principles: (1) the stabilisation of wine via the creation of a nutrient desert (i.e. through fermentation to dry and the exhaustion of nutrients, especially nitrogen, fermentable sugar and micronutrients); (2) the creation of a 'microbial balance' at the end of the winemaking process and its preservation by not sterile filtering; and (3) the 'aromatic integration' of ethylphenol into the tannic structure of the wine via micro oxygenation.

Smith suggests a paradigm shift whereby microbial activity, including that of Brett cells is to be fully reintegrated in the winemaking process. Instead of tempting to control and at best eradicate Brett, to make 'clean, sterile wines of appealing fruit character' (2015, n.p.), Smith proposes an inverse approach where 'the development of fully-evolved, structured wines for which profundity rather than varietal purity is the goal' (2015, n.p.). To implement such an approach, he recommends that Brett should normally be controlled through natural competition with other microbial agents. Also, once he claims that the 'point of most red wine is to evolve away from its original simple plant characters of berries and herbs into something richer and warmer', fermentative aromas and flavours including those produced by Brett must be considered as part of the 'rich underlying structure which supports and integrates developing flavours into a coherent single voice' (Smith, 2015, n.p.).

While the first and, to a certain degree also the second, principle outlined above may make sense from an oenological perspective, the third is far less clear, and its functioning less certain. Smith (2015, n.p.) argues that:

> 'the aromas of varietal vegativity, oak and microbial activity can be integrated into a good phenolic structure. The finer the colloids in such a structure, the more surface area will be available for aromatic integration. Refinement of tannin colloids requires oxygen […] without encouraging microbial growth. Wines of proper ripeness and good extraction afforded early structural refinement can carry many times the supposed "threshold" of 400 ppb of the Brett metabolic marker 4-ethly-phenol without

apparent aromatic expression. Indeed, the nuances added to the flavour impression in the nose and by mouth imparted by a Brett manifestation in these conditions are likely to be quite positive. If such a wine is made without any sulphites, the ensuing complex microbial activity can enhance terroir expression of cherries, wild flowers and garrigue without objectionable intrusion of spoilage characteristics, resulting in wines of greatly enhanced profundity and soulfulness.'

Microoxygenation is a technology by means of which very small quantities of pure oxygen are infused into the wine via a metal, acrylic or ceramic diffuser. It is practiced at different stages of the winemaking process, during fermentation, before malolactic fermentation and during aging. The technology has been employed commercially in France since 1991 (cf. Anli et al., 2012). Its pioneer was Patrick DuCournau whose idea was to simulate – and accelerate – the slow oxygenation of red wines taking place during barrel aging. The fine pores of the barrel wood allow oxygen to pass into the wine at a very low rate, between 1-4 mg/L/month according to the oak type and condition (Jones et al. 2004). This leads to the production of acetaldehyde, formed chemically through the oxidation of ethanol. Acetaldehyde, in turns, can crosslink tannins and anthocyanins, and create larger molecules or polymers.

Microoxygenation simulates this process, yet by adding larger amounts of oxygen, up to threshold levels of 60mg/L/month in a tannin and pigment rich red wine over periods of 2 to 4 weeks before malolactic fermentation and up to 10ml/L/month over periods of 2 to 5 months during wine aging (Atanasova et al. 2002; Anli et al., 2012). It is assumed that the acetaldehyde made available by that means plays a crucial role for the formation of larger tannin colloids, with a supposedly less astringent mouth feel (because of their lesser ability to bind proteins in the saliva present in the mouth of a wine consumer), and also of tannin-anthocyanin colloids which enhance and stabilise colour. The main rational of using microoxygenation is economical: the slow process of oxygenation during barrel aging can be simulated and accelerated in steel tanks. (Microoxygenation is also applied during fermentation, usually with the aim to help the growth of yeast cell populations and avoid stuck fermentations.)

Different recent studies seem to confirm the hypothesis about the effect of microoxygenation on colour, while the effect on astringency is less certain (cf. Du Toit et al., 2006; Blaauw, 2009; Arapitsas et al., 2012; Anli, 2012). A main argument against the polymerisation hypothesis is that polyphenol polymerisation is chemically a far more complex phenomenon that involves other compounds and thus cannot be seen as isolated process. Also, although

already a bit dated, Jones et al.'s work (2004) stresses the absence of actual proof of the effect of microoxygenation on the enhancement of fruity flavours, as microoxygenation users and equipment providers seem to claim it (cf. Malfeito-Ferreira et al., 2001). Smith (2013), a champion of this technology in the US, argues – in line with what has hitherto been assumed by most academics – that controlled microoxygenation can build structure and longevity, and enhance mouth feel. Moreover, as mentioned above, he stresses that such wines can 'carry many times the supposed "threshold" of 400 ppb of the *Brett* metabolic marker 4-ethly-phenol without apparent aromatic expression'.

2.6 Research perspectives

The review of the current knowledge about *B. bruxellensis* provides a series of key information: Brett is a yeast highly diverse in its biological expressions and oenological effects, with differentiated actions according to strains, berry variety, wine structure, and even geography. It is a dangerous factor for wine spoilage and its distinctive bretty aromas are not for mainstream mass-produced everyday wine. Saying that, many high value wines clearly have a bretty character, which seems indicative for the socially and culturally acquired nature of wine consumer taste. Further research is needed (1) to better understand this social nature and value associated with bretty wine; (2) to identify selected, geographically distinct, and potentially noble Brett strains that could be used in industrial winemaking, for instance through the controlled inoculation of smaller batches with selected Brett strains and back-blending; (3) to create protection measures of local environments (including endemic Brett strains) against global Brett contaminations and epidemics, namely via the trade in second hand cellar material and equipment; (4) to test the hypothesis of aromatic integration of Brett aromas as suggested by Smith.

To our knowledge it has not been demonstrated anywhere that ethylphenol – a neutral molecule – can be bound, in any way, into the tannic structure of a wine. Subsequently, we suggest a hypothesis alternative to that by Smith. Building on Morata et al.'s work on the formation of vinylphenolic pyranoanthocyanins, we suggest that the 'aromatic integration' that Smith talks about takes place mainly after the first step of the twofold *B. bruxellensis* metabolism of hydroxycinnamic acid. It is reducing and transforming the vinyl compounds: 4-vinylphenol, 4-vinylguaiacol and 4-vinylcatechol and, by that means, decreasing their availability for further metabolic conversion into ethylphenols. At the same time, this formation of pyranoanthocyanins is likely to enhance and stabilise the wine's colour. Observing that this chemical reaction requires oxygen, it is assumed that microoxygenation will facilitate and accelerate this process (Fig. 4, left side). In addition, as suggested in the

academic literature it will also form acetaldehyde crosslinking anthocyanins and tannins, leading to colour stabilisation and intensification (cf. Fig 4, right side).

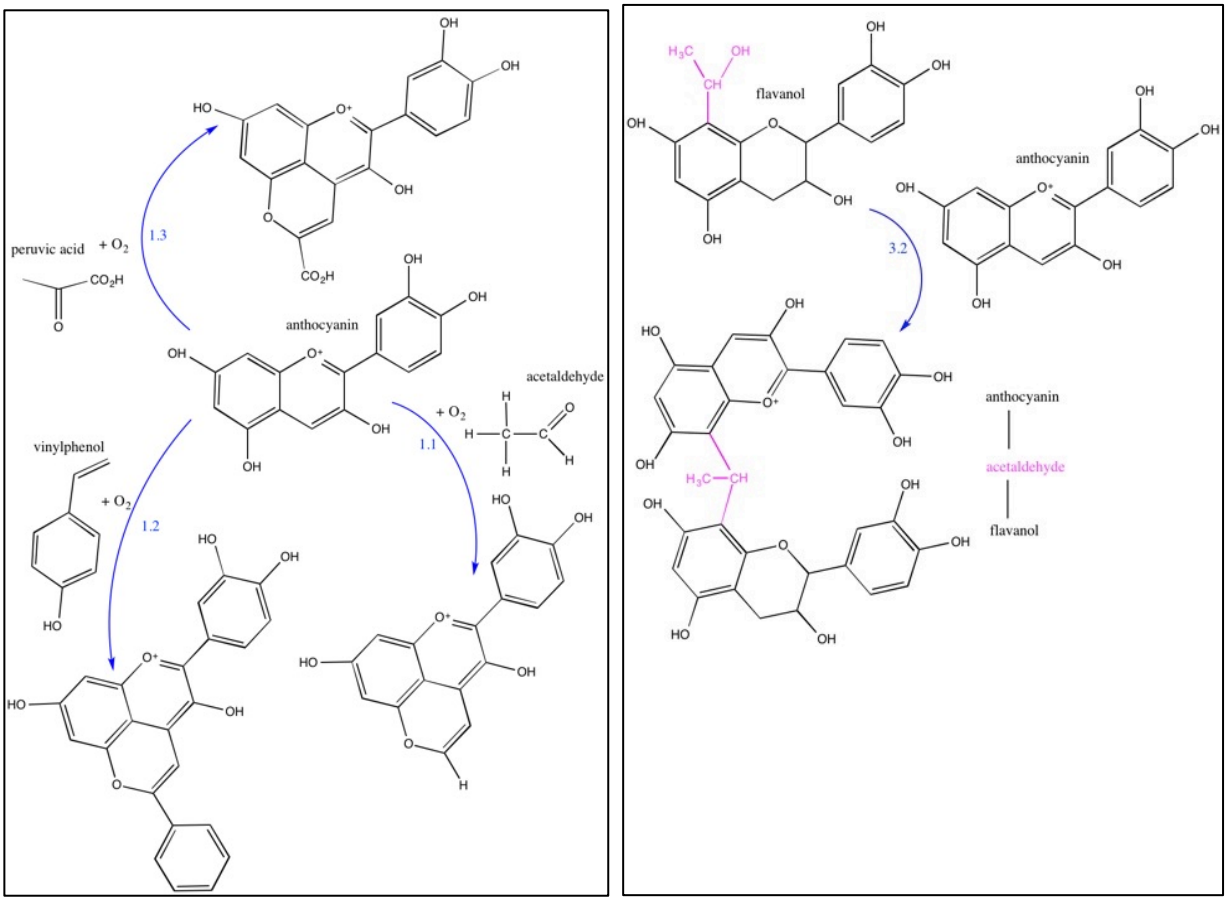

Fig. 4: Formation of pyranoanthocyanins (left diagram) and acetaldehyde-bridged anthocyanin-flavanol molecules (right diagram)

The next chapter will outline the research set-up, materials and methods that allowed this to happen.

Chapter 3. Materials and methods

To test the two alternate hypotheses outlined in the previous chapters, a group of five 25-L tanks with previously fined and micro-filtered Rheingau red wine was spiked with different concentrations of vinyl and ethyl compounds and subjected to microoxygenation at around 30 mg/L/month for ten days. A further group of 5 tanks with identical preparations was used as a control, without microoxygenation. With one complete repetition of this set-up, the experiment involved a total of 20 tanks.

3.1 Base wine

The base wine was a blend of 2013 red *Vitis vinfera* (ExE) QBA wines from the German Rheingau region. The untreated and still unprepared base wine had a pH of 3,4, an alcohol content of 10,50% vol., 1,1 g/L of residual sugar, 5,8 g/L of total acidity, 0,4 g/L of volatile acidity, 12 mg/L of free SO_2, 93 mg/L of bound SO_2 and 1237mg/L of total phenolic content. The wine had undergone malolactic fermentation and had since been stored in a steel tank (for about 12 months). When receiving the wine it contained 2 mg/L of dissolved O2. It came in a 1000L PVC plastic transport tank with an available content of 640 L.

With reference to the norms for sensorial analysis by the Wine & Spirit Education Trust[2] (2012), the wine was clear, with a medium intense ruby aspect and no legs. In the nose, it was clean, with highly volatile medium-plus intense red and black fruit aromas. In the mouth, it had medium plus acidity, medium alcohol, medium body, and medium intense red and black fruit aromas. The wine was fresh and fruity, with a good flavour-acidity balance.

To prepare the wine for the experiment, it was first fined with bentonite to eliminate proteins and then filtered with K100 (Seitz) plates with a pore size of 1,5 µm to remove all yeasts. For reference, cells of *B. buxellensis* have a size between 5 and 7 µm; those of *S. cerevisiae* according to their form – haploid or diploid – have a size between 4 and 6 µm (Sherman,

[2] In the course of his graduate studies at the University of Geisenheim, the author has undergone training and an examination by the Wine & Spirit Education Trust equivalent to the WSET Level 3 Certificate.

1991). The microfiltration was judged sufficient to assume the removal of all viable yeast cells. The later microbiological examination of the wine at the beginning and end of the experiment revealed the presence of viable cells of *S. cerevisiae*, fungi and bacteria, but the absence of *B. bruxellensis*. It is likely that a contamination with fungi and bacteria occurred when spiking the wines with tannins (cf. next paragraph): the later microbiological examination of the tannins (dissolved in a sterile 0,9 % NaCl-solution) revealed 14 fungi and bacteria. The cells of *S. cerevisiae* may have entered the wine during handling. Learning from this experience, it is recommended for any future repetitions of this experiment to sterilise the wine and its additives with more care.

To test the hypothesis of chemical reactions between flavonoids and different vinyl and ethyl Brett metabolites in the presence of oxygen, the wine was spiked both with oenological tannins and with ethyl and vinyl compounds. To produce more significant results, it had initially been suggested to increase the wine's anthocyanin concentration; yet for logistic and economical reasons oenological anthocyanins could not be made available. Through the addition of 763mg/L (corresponding to 76g/HL) of a combination of ellagic and proanthocyanidic tannins the total phenolic content was increased to approximately 2000mg/L, which corresponded to an approximate ratio of 500mg/L of anthocyanins (a reference value for similar Rheingau wines, cf. Fischer & Durner, 2010) for 1500mg/L of tannins in the wine. The product used to spike the wine was Laffort's Tannin VR Supra, a combination of proanthocyanidic tannins derived from grapes and ellagic tannins from oak with the double oenological aim to condense anthocyanins and stabilise colour, and to add phenolic structure to the wine by increasing its total phenolic content. The tannins were mixed into 5L of wine and the mixture into the plastic transport tank with a mechanical mixer. The wine then reposed for 3 days.

3.2 Vinyl and ethyl concentration with which to spike the test wine

To achieve its objectives, the wine needed to be spiked with different concentrations of ethyl and vinyl compounds, above the respective sensorial thresholds. Because earlier works on such thresholds (cf. Chapter 2) show that the specific style and structure of each given wine influences these relative thresholds, a preliminary taste panel was run to determine the specific thresholds for the wine used in this experiment. Based on reference values form the academic literature (Chatonnet et al., 1992; Bisson et al. 2007; Eder, 2010, Botha, 2010), different series of spiked wines with increasing concentrations of ethyl and vinyl compounds and combinations of both were subjected to a panel of 10 trained tasters. The tasters were instructed to swiftly smell and then briefly describe each wine and judge its pleasance on a

scale from 1 to 5. Each series of wines was attributed a line in a table on an A4 sheet, with one box for each wine for wine descriptors and a number indicating how pleasant the wines were (cf. Fig. 5). The tasters were given base wine as control, against which to compare the other wines (corresponding to wine number 0 on the sheet). They were also explained to focus on dominant secondary aromas, apart from the wine's strong fruit aroma.

0			
1 (670 µg/L EP)[3]	2 (870 µg/L EP)	3 (1000 µg/L EP)	4 (1270 µg/L EP)
5 (100 µg/L EG)	6 (125 µg/L EG)	7 (160 µg/L EG)	8 (185 µg/L EG)
9 (200 µg/L VP)	10 (400 µg/L VP)	11 (600 µg/L VP)	12 (800 µg/L VP)
13 (60 µg/L VG)	14 (90 µg/L VG)	15 (120 µg/L VG)	16 (150 µg/L VG)
17 (600 µg/L VP + 200 µg/L VG)	18 (800 µg/L VP + 300 µg/L VG)		
19 (620 µg/L EP + 185/L µg EG)	20 (870 µg/L EP + 185/L µg EG)		
21 (600 µg/L EP + 200 µg/L EG + 600 µg/L VP + 200 µg/L VG)	22 (600 µg/L EP + 200 µg/L EG + 800 µg/L VP + 300 µg/L VG)	23 (700 µg/L EP + 200 µg/L EG + 800 µg/L VP + 300 µg/L VG)	

Fig. 5: Sheet used for the preliminary tasting

Based on this preliminary tasting, values for the detection thresholds and 'sweet spots' for different vinyl and ethyl compounds could be identified. The detection thresholds were: 670 µg/L for ethylphenol,160 µg/L for ethylguaiacol, 600 µg/L for vinylphenol and 150 µg/L for

[3] All values in brackets in the table denote the concentrations of vinyl and ethyl additions; these were not shown on the original sheets used during the tasting. EP = ethylphenol, EG = ethylguaiacol, VP = vinylphenol, VG = vinylguaiacol.

vinylguaiacol. For the combinations of compounds, a difference with regard to the control was respectively detected at the respectively lowest concentrations of additives (cf. Fig 5, wines 17-23). Unexpectedly, the increase of the concentration of additives did not lead, apparently, to a progressive increase of aroma intensity or linear curves of aroma sweet spots, but to significant shifts between aroma descriptors and aroma preferences (for detailed results, cf. chapter 4).

3.3 Experiment and tank set-up

The observations on the sensorial detection thresholds and the apparently non-linear sweet spot curves were used to define the experiment and tank set-up for the actual experiment.

In two tanks, vinyl and ethyl compounds were respectively added in concentrations slightly above the sensorial detection threshold, at a respective ratio of 800µg/L of phenol and 200µg/L of guaiacol compounds. In two further tanks was added a combination of all vinyl and ethyl compounds, respectively in concentrations of 800µg/L and 1600 µg/L of phenol and 200µg/L and 400 µg/L of guaiacol, to simulate medium and very strongly bretty wines. One tank was not spiked to serve as control. The entire set-up was quadruplicated to be able to test a series of tanks with microoxygenation against one without, and to run one full repetition (cf. Table 4).

Table 4. Experiment and tank set-up

Tank number	Treatment	4-vinylphenol	4-vinylguaiacol	4-ethylphenol	4-ethylguaiacol
1	MOX	800µg/L	200µg/L	0	0
2	MOX	800µg/L	200µg/L	0	0
3	MOX	0	0	800µg/L	200µg/L
4	MOX	0	0	800µg/L	200µg/L
5	MOX	800µg/L	200µg/L	800µg/L	200µg/L
6	MOX	800µg/L	200µg/L	800µg/L	200µg/L
7	MOX	1600µg/L	400µg/L	1600µg/L	400µg/L
8	MOX	1600µg/L	400µg/L	1600µg/L	400µg/L
9	MOX	0	0	0	0
10	MOX	0	0	0	0
11	No MOX	800µg/L	200µg/L	0	0
12	No MOX	800µg/L	200µg/L	0	0
13	No MOX	0	0	800µg/L	200µg/L
14	No MOX	0	0	800µg/L	200µg/L
15	No MOX	800µg/L	200µg/L	800µg/L	200µg/L
16	No MOX	800µg/L	200µg/L	800µg/L	200µg/L
17	No MOX	1600µg/L	400µg/L	1600µg/L	400µg/L
18	No MOX	1600µg/L	400µg/L	1600µg/L	400µg/L
19	No MOX	0	0	0	0
20	No MOX	0	0	0	0

For the experiment, 25,4L glass balloons were filled to full and closed with rubber stoppers. The tanks had been cleaned and sterilised before the experiment. The wine was carefully

racked from its plastic transport tank into these glass tanks, avoiding additional oxygen uptake. The vinyl and ethyl compounds, previously prepared from dry substance in 5% and 10% solutions, were added with the help of an Eppendorf pipette and carefully stirred into the wine with an inox spoon (50 times). Care was taken to consider the initially present concentration of ethylphenol in the base wine in the calculation. The oxygen levels measured before and after stirring went up by approximately 10%, from 0,40 mg/L to 0,43 mg/L.

The experiment was carried out in the barrel cellar of the oenology department of the University of Geisenheim, which offered constant temperatures at 15 degrees C, constant humidity and very low light. For practical and technical reasons, the entire setup was moved to a cellar 2 degrees warmer 3 days after the start of the experiment.

Fig. 6: Tank set-up in the cellar of the University of Geisenheim

3.4 Microoxygenation in small tanks

According to the academic literature, a heavy red wine is able to break up between 50 and 70 mg of oxygen per L/month at 15 degree Celsius (cf. Chapter 2). In practice, this is a rule-

of-thumb value that actually varies from wine to wine. The amount of added oxygen should therefore be calibrated to the specific reductive capacity of each specific wine.

To obtain a value for this specific reductive capacity of the wine used in this experiment, the unprepared base wine was saturated with oxygen, by means of prolonged heavy shaking. The oxygen reduction was then measured within 1-minute intervals over 17 hours at an ambient temperature of 15,5 degrees Celsius, using an oxygen sensor spot stuck inside two of the glass balloons (for more details on this technology, cf. section below on oxygen measurement). The data series generated showed a close-to linear oxygen reduction at a rate of 0,07 mg/L/hour, 1,68 mg/L/day or 51 mg/L/month (cf. Fig 7).

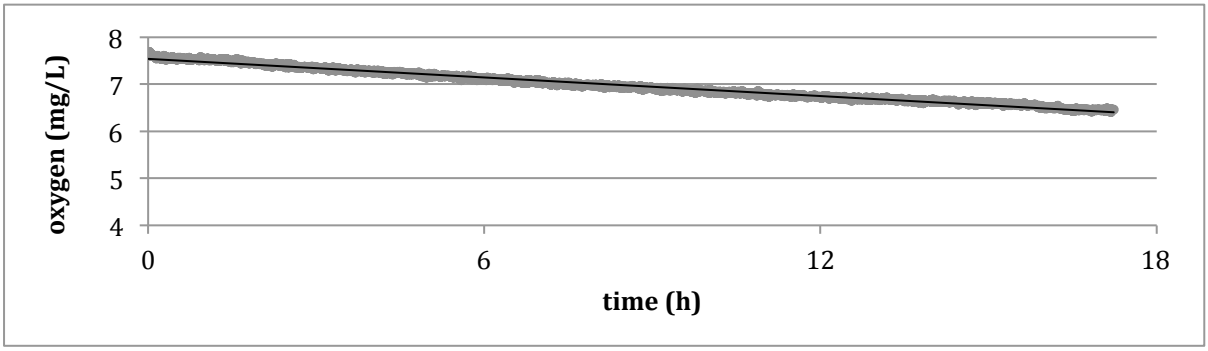

Fig. 7: Oxygen reduction in the unprepared base wine

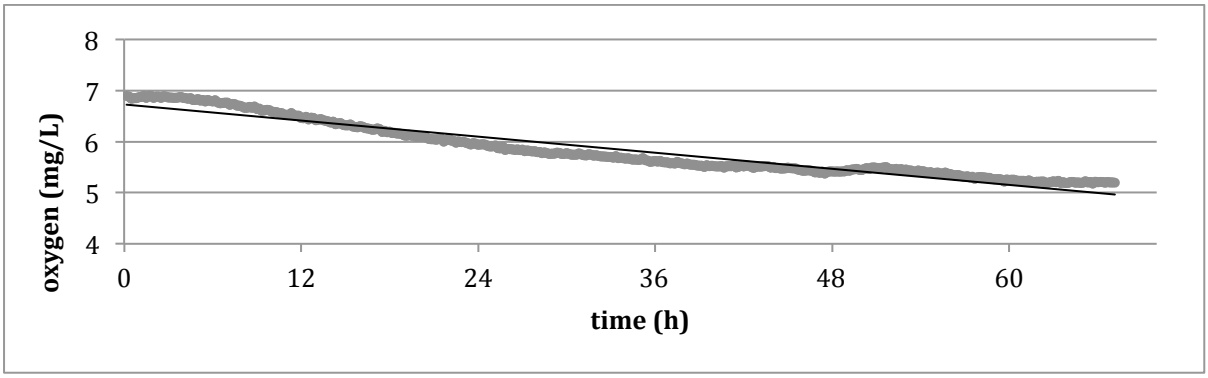

Fig. 8: Oxygen reduction in the filtered and tannin-spiked test wine

The experiment was repeated with the filtered and tannin-spiked wine over 3 days, with a measure every 10 minutes at an ambient temperature of 17,3 degrees Celsius. Here the values were slightly higher showing an oxygen reduction of 0,09 mg/L/h, 2,16 mg/L/day or 65 mg/L/month, again following a more or less linear trend (cf. Fig 8).

If the aim of microoxygenation is to supply just that amount of oxygen that the wine is able to break-up (plus the part that evaporates). The values measured here corresponded to oxygenation levels between 1,78 and 2.29 mg/h or between 42,67 and 54,86 mg/day for a

25,4 L tank (cf. table 5). Technologically, it was not possible to create a research set-up that would permit to provide a constant controlled supply of such low oxygen levels.

Table 5: Average oxygen reduction capacity per minute, hour and day

	Base wine	Filtered and tannin-spiked wine	25,4 L of filtered and tannin-spiked wine
per h	0,07 mg/L	0,09 mg/L	2,29 mg
per day	1,68 mg/L	2,16 mg/L	54,86 mg
per month	50,40 mg/L	64,80 mg/L	1645, 80 mg

To resolve this problem, the technology eventually adopted was to apply the total daily oxygen supply via shorter oxygen dosages of just above 1mg/L twice a day (to reach the 2,16 mg/L/day that the wine was able to reduce). The rationale was that the wine would in this case dissolve and store the oxygen for twelve-hour periods during which it would be progressively broken up.

The set-up consisted of a bottle of liquid oxygen linked to a system of two sequential regulators, the first manual (adjusted to 0,2 bar) and the second controlled by a computer programme (adjusted to 0,9 bar and a nominal flow rate of 65 ml/second), and a ceramic diffuser. Before the experiment different diffusers were compared and the one with the – seemingly – most homogenous air bubble production selected. In a larger tank, the atmospheric pressure related to the height – 1bar for every 10m – would have needed to be considered. In this case, the 25L tanks measured 60 cm in height, which is negligible in terms of additional pressure (corresponds to 0,06 bar).

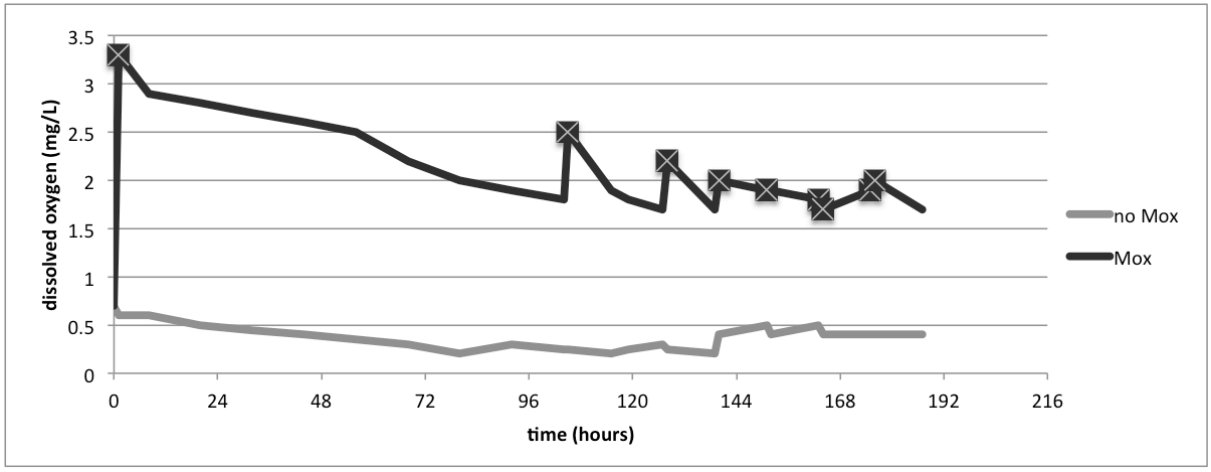

Fig. 9: Disolved oxygen in tanks with and without microoxygenation application (crossed boxes correspond to MOX applications)

To determine the appropriate duration for the bi-daily oxygen applications, a series of tests with increasingly longer periods of microoxygenation was carried out, and oxygen measured before and after. After an initial calculation mistake (leading to an oxygen overexposure in

the beginning of the actual experiment), the application time was set to 10 seconds twice a day, with adjustments of +/- 3 seconds whenever the oxygen levels in a particular tank varied significantly. After this readjustment of the microoxygenation application periods, the technology worked as expected (cf. Fig 9.).

To systematize the set-up and approach, an experiment research protocol was elaborated to define the series of tasks of the bi-daily microoxygenation applications (cf. Fig 10).

1. Turn on pc and check connections with regulator
2. Turn on oxygen sensor spot computer and oxymeter
3. Get two buckets of water and make 1:7 alcohol solutions.
4. Mount diffuser, open oxygen, check oxygen is open
5. Open regulator app, find diffusor
6. Open excel in the background, with sheets prepared Get timer to 10 se. Open wine tank.
7. Measure O2 in tanks 10 and 20, write up result
8. Start button in regulator app
9. Get diffuser in the wine and start timer
10. When timer is finished, take diffusor out and put in water bucket 1.
11. Put diffusor in alcohol (10 sec) then in water bucket (20 sec)
12. Stop regulator app, copy-paste data in excel, open next excel sheet
13. Close tank
14. Open tank.
15. Get timer ready and put diffuser in next tank

End

1. At last tank, same diffusor cleaning.
2. Close O2.
3. Measure O2 in tank 10.
4. Take off diffusor tube and connect to compressed air (very carefully). Apply air to dry. Put back in glass container.
5. Save excel file and copy to USB.
6. Shut down PC. Unplug electricity.
7. Clean water and alcohol buckets, and cellar floor if necessary.
8. Return car outside cellar. Switch off lights.

Fig. 10: Experimental research protocol for the application of microoxygenation

3.5 Oxygen measurement

The amount of oxygen dissolved in the wines was monitored by means of an electric oxygen meter. Additionally, oxygen sensor spots were clued at the inside of two of the tanks. (Different oxygen levels provoke the sensor spots to reflect light waves differently, hence providing a good proxy for actual dissolved oxygen levels.) The technology is non invasive and does not make it necessary to open a tank or to have physical contact with the measured medium. The downside in this specific experiment was that the two sensor spots

used saturated with oxygen much faster than the wine, when microoxygenation equipment was used, hence not providing a reliable measure after the oxygen applications. The sensor spots got back to normal within a few hours. To resolve this issue, in addition to the oxygen sensor spots a classical dissolved oxygen meter was used.

Gas chromatography

Stir bar sorptive extraction (SBSE) and gas chromatography with thermodesorption (TDU, Gerstel Germany) followed by mass spectrometric detection were used to measure the concentrations of 4-ethylphenol, 4-ethylguaiacol and 4-vinylguaiacol (according to Sponholz et al. 2001 and modified by Stiehl and Rauhut (unpublished)). The analysis of 4-vinylphenol (and 4-vinylguaiacol) used an adapted protocol according to Rapp et al. (1994) and modified by Ebert and Rauhut (unpublished), which produced significantly equal results for 4-vinylguaiacol, measured by both methods.

3.6 Spectrophotometry

Spectrophotometry (Cadas 200, Dr Lange, Germany) was used to measure the colour intensity and nuance (hue) of the test wines before and after the experiment, with a λ-scan with a spectrum range of 380-770 ηm. All samples were previously centrifuged at 14500 rpm for 5 minutes.

3.7 Microbiological methods

To verify the presence (or absence) of fungi, yeasts and bacteria in the test wines, microbiological tests were carried out using a standard protocol. This consisted in the membrane filtration (at 0,45 µm) of 100 ml samples. The filtrate was then cultivated in two different media at 27° C for one week. The filters were then counted out and representative samples of particular cultures taken off and analysed both microscopically and through microbiological cultivation and FTIR. Additionally, 'Sniff' Brett' (Dolmar, Spain) was used to verify the presence of viable Brett cells in the wine.

3.8 Sensorial analysis

Three sets of sensorial analysis were carried out: (1) triangular tests to verify if tasters detect sensorial differences between samples subjected to microoxygenation and their respective control; (2) preference ranking tests to compare how different samples are judged against each other and if significant differences in the overall appreciation can be verified; (3) a multi-series descriptive test with an open Likert scale from 0 to 9, to measure the detected

intensities of different aroma groups in each wine and its overall appreciation in terms of pleasance.

The samples and test sheets were prepared and analysed with the help of the sensory analysis solution Fizz (Biosystèmes, France).

The triangular test and the first ranking test were carried out with a panel of 15 grad students of the Vinifera Euromaster and Vitis Vino programme hosted at the University of Geisenheim, all trained in sensory analysis. The second ranking and descriptive tests were carried out with five distinct taste panels panel: (1) Vinifera Euromaster and Vitis Vino students of the University of Geisenheim; (2) German oenology students of the University of Geisenheim; (3) Portuguese oenology students of the University of Lisbon; (4) French oenology students of the University of Bordeaux and (5) Swiss oenology students of the University of Changins. Once the results from the Swiss panel arrived only in the very last days of writing this work, they could not be included in the analysis.

In the descriptive test, the selection of the aroma groups analysed was based on the results of the preliminary taste-panel, which asked the tasters to suggest descriptors for each wine (cf. section 'Vinyl and ethyl concentration with which to spike the test wine' above, in this chapter). It was also based on the main groups of the Brett aroma wheel proposed by Bisson and Joseph (cf. Fig. 3 in chapter 2). The descriptive test included an additional part with multiple-choice questions; these were aroma subgroups within each main aroma group that tasters were instructed to select whenever they gave a score above 6 to the aroma main group. The descriptive test also included social criteria to analyse if the attributed wine descriptions and preferences varied as a function of age, gender and cultural background or training of the tasters.

A briefing note that explained the panel administrator how to prepare the wines and how to instruct the tasters accompanied all tastings. All tastings were carried out in dedicated professional taste rooms (with exception to Portugal, where the tasting took place in a classroom yet with similar conditions).

Chapter 4. Results

4.1 Research set-up worked – repetitions are consistent

The concentrations of ethyl and vinyl compounds added before the experiment – measured in the beginning, during and at the end of the experiment in each tank and its corresponding control – were significantly equal. The analysis of these values by a Student paired test produced values very significantly above 0,05 (cf. Table 6), which indicates that the experiment has been carried out under properly controlled and repeatable conditions.

Table 6: Student t-test comparing vinyl and ethyl concentrations in each tank and its control the beginning, during and at the end of the experiment

Paired samples	**t-test (p-value)**	**Initial concentrations**	**Treatment**
Tanks 1 & 2	0,281619614	800 µg/L vinylphenol 200 µg/L vinylguaiacol	MOX
Tanks 3 & 4	0,142439753	800 µg/L ethylphenol 200 µg/L ethylguaiacol	MOX
Tanks 5 & 6	0,075276248	800 µg/L vinylphenol 200 µg/L vinylguaiacol 800 µg/L ethylphenol 200 µg/L ethylguaiacol	MOX
Tanks 7 & 8	0,302922022	1600 µg/L vinylphenol 400 µg/L vinylguaiacol 1600 µg/L ethylphenol 400 µg/L ethylguaiacol	MOX
Tanks 9 & 10	0,182051767	base wine 720 µg/L ethylphenol 120 µg/L ethylguaiacol	MOX
Tanks 11 & 12	0,263005927	800 µg/L vinylphenol 200 µg/L vinylguaiacol	No additional oxygen
Tanks 13 & 14	0,412392832	800 µg/L ethylphenol 200 µg/L ethylguaiacol	No additional oxygen
Tanks 15 & 16	0,067172442	800 µg/L vinylphenol 200 µg/L vinylguaiacol 800 µg/L ethylphenol 200 µg/L ethylguaiacol	No additional oxygen
Tanks 17 & 18	0,073828585	1600 µg/L vinylphenol 400 µg/L vinylguaiacol 1600 µg/L ethylphenol 400 µg/L ethylguaiacol	No additional oxygen
Tanks 19 & 20	0,141866909	base wine 720 µg ethylphenol 120 µg ethylguaiacol	No additional oxygen

4.2 No effect of microoxygenation on ethylphenol concentration

In all tanks, the start and end-of-experiment concentrations of ethylphenol (Fig 11) and ethylguaiacol (Fig. 12) remained stable (p-values of t-test of 0,248449597 and 0,155397263 respectively). In other words, neither reduction nor production of ethyl derivatives could be observed.

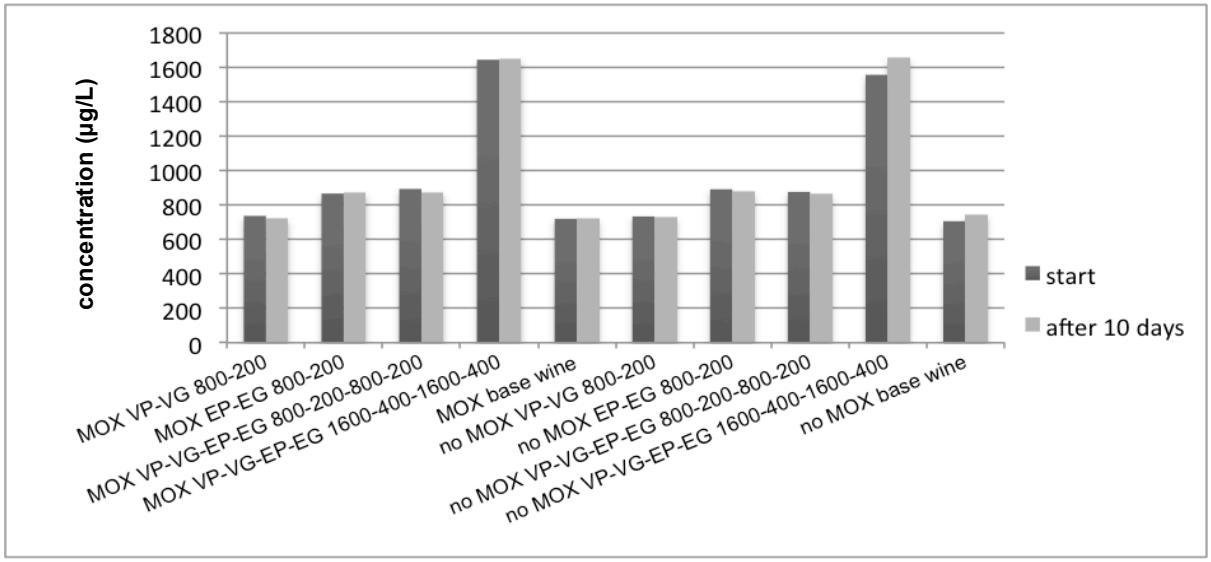

Fig. 11: Comparison of ethylphenol concentrations at the start and after 10 days of experiment

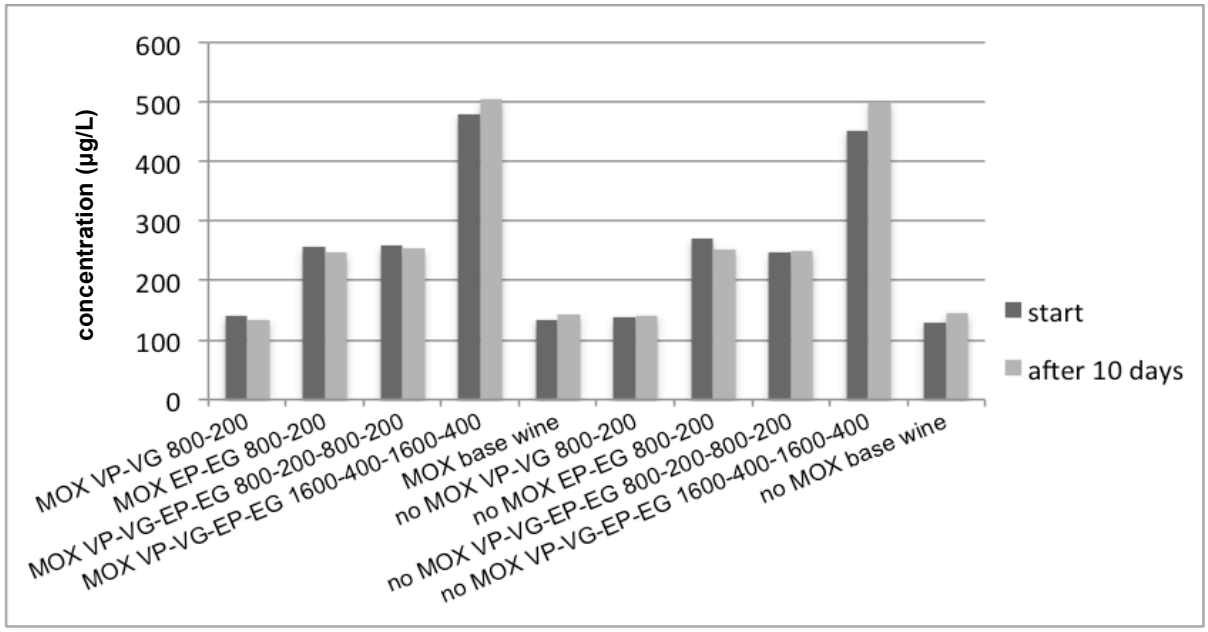

Fig. 12: Comparison of ethylguaiacol concentrations in the test wines at the start and after 10 days of experiment

4.3 Microoxygenation accelerates reduction of vinyl compounds

The measure of the concentrations of vinylphenol and vinylguaiacol 7 and 11 days after the start of the experiment showed a significant reduction of up to 80% in all tanks containing these additives (cf. Fig. 15). It is striking that the start values are, at times, quite far away from the target values for the experiment, which did, however, not alter the general observation of a systematic reduction of these compounds.

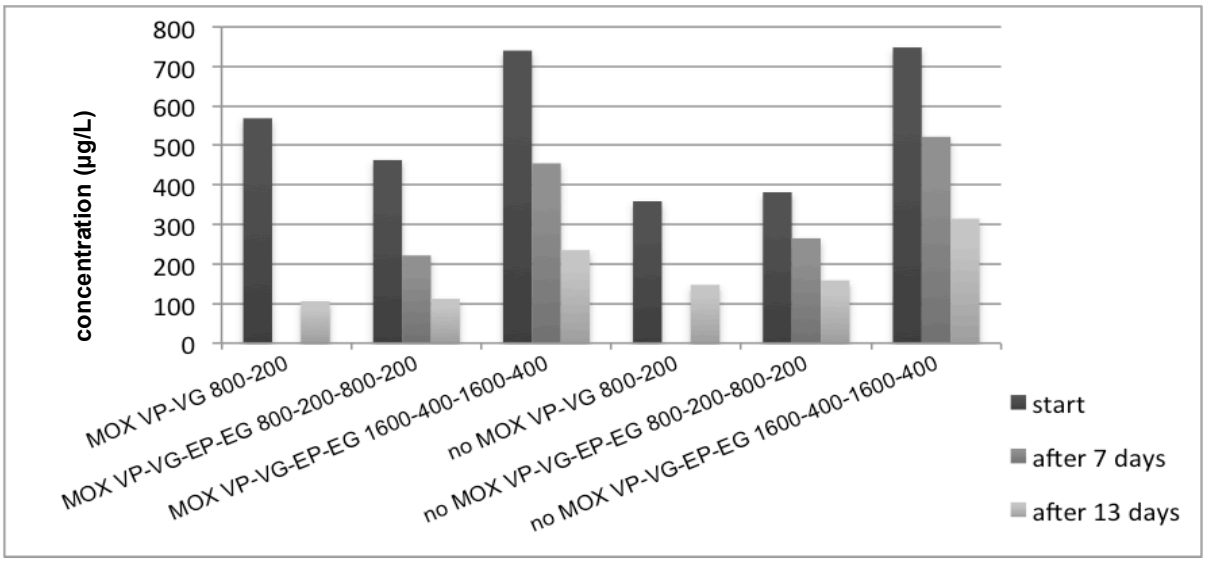

Fig. 13: Comparison of vinylphenol concentrations in the test wines at the start and after 7 and 13 days of experiment

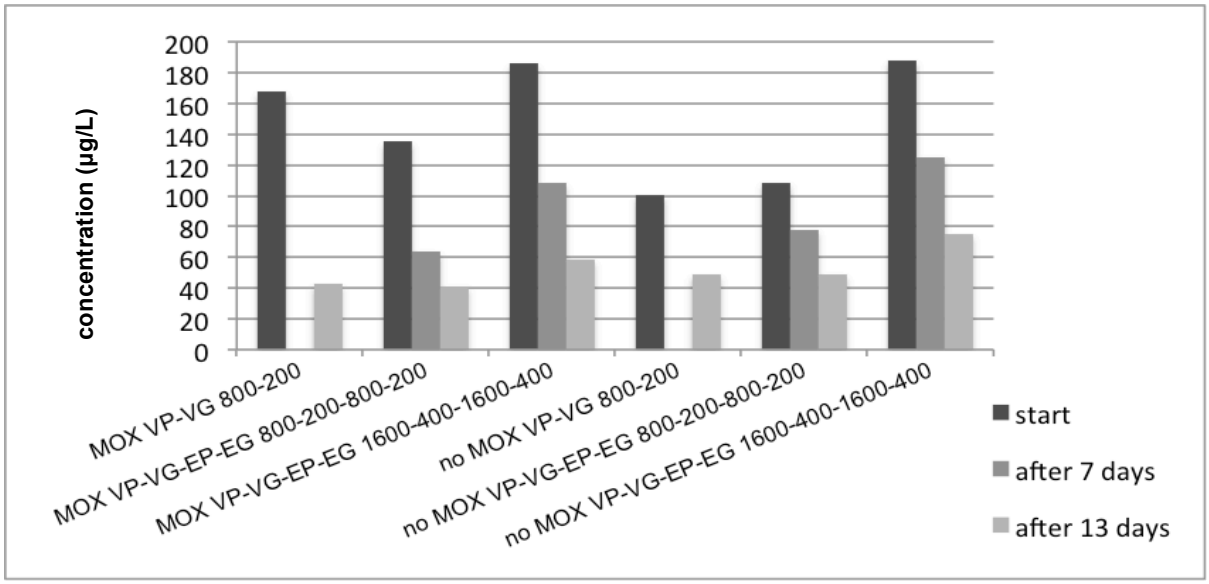

Fig. 14: Comparison of vinylguaiacol concentrations in the test wines at the start and after 7 and 13 days of experiment

When comparing the rates of vinylphenol and vinylguaiacol reduction in tanks with, and without microoxygenation, a very clear difference can be observed: the wines subjected to microoxygenation experienced a significantly faster rate of reduction. In this sense, oxygen helps to break-up vinyl compounds.

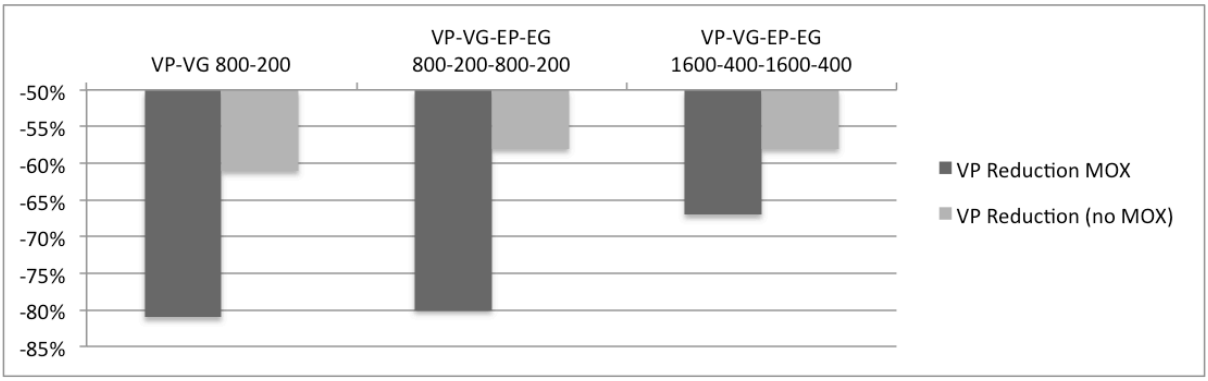

Fig. 15: Effect of microoxygenation on vinylphenol reduction after 13 days of experiment

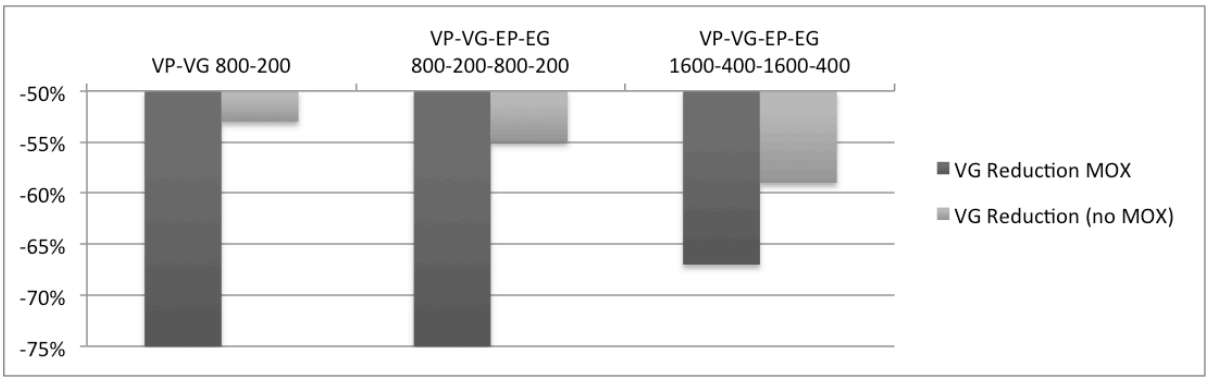

Fig. 16: Effect of microoxygenation on vinylguaiacol reduction after 13 days of experiment

4.4 Tannin addition and higher temperature accelerate vinyl reduction

Based on the observed reduction of vinylphenol in the presence of oxygen, a follow-on test was carried out to test the influence of the tannins that had been added to the base wine and of different temperatures on the rate of this reduction. Two series of a model wine and a colour-intense red wine (with low free SO2) were variably spiked with 1mg/L of vinylphenol and with a combination of 1mg/L of vinylphenol and 763 mg/L of tannins (the same tannins and tannin concentration that was used in the initial experiment). The series were kept for three days at 20 degrees and at 5 degrees Celsius respectively and the vinylphenol reduction was measured via gas chromatography.

The results show that in both types of wine, the reduction of vinylphenol was significantly increased in the presence of added tannins and at higher temperature.

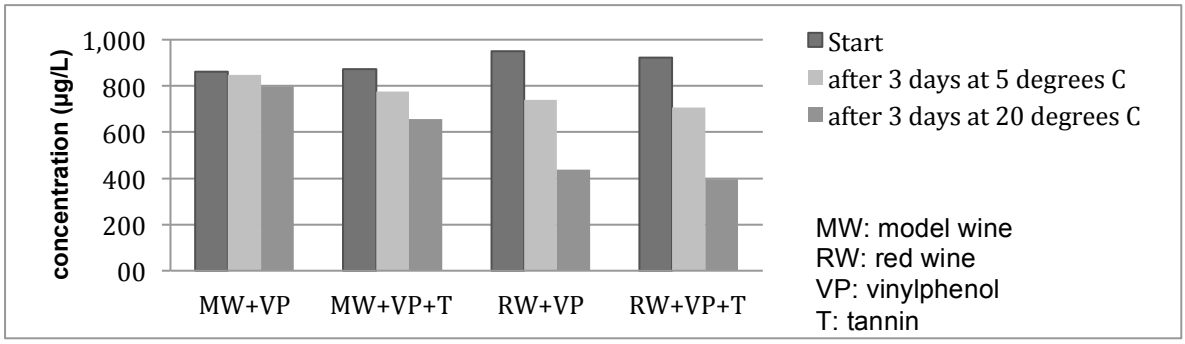

Fig. 17: Vinylphenol reduction as a function of added tannins and different temperatures

4.5 Microoxygenation provokes gain in colour intensity and slight shift toward red-orange hues

Colour intensity was calculated by adding for each sample the photo-spectrometric values measured at 420 nm (indicating yellow), 520 nm (indicating red), and 620 nm (indicating violet). The hue value was calculated by dividing for each sample the photo-spectrometric values measured at 420 nm (yellow) by those measured at 520 nm (red). The ANOVA tests for the intensity and hue values of the two series of tanks (subjected and not to microoxygenation) showed no significant differences within each series (F-values respectively of 0,1576, 0,0691, 0.1310 and 0,069118 with respective p-values consistently above 0,05), but significant differences between them. This means that microoxygenation had a significant impact upon these values, while the different ethyl and vinyl additives (that defined the differences within these series) did not have any such impact.

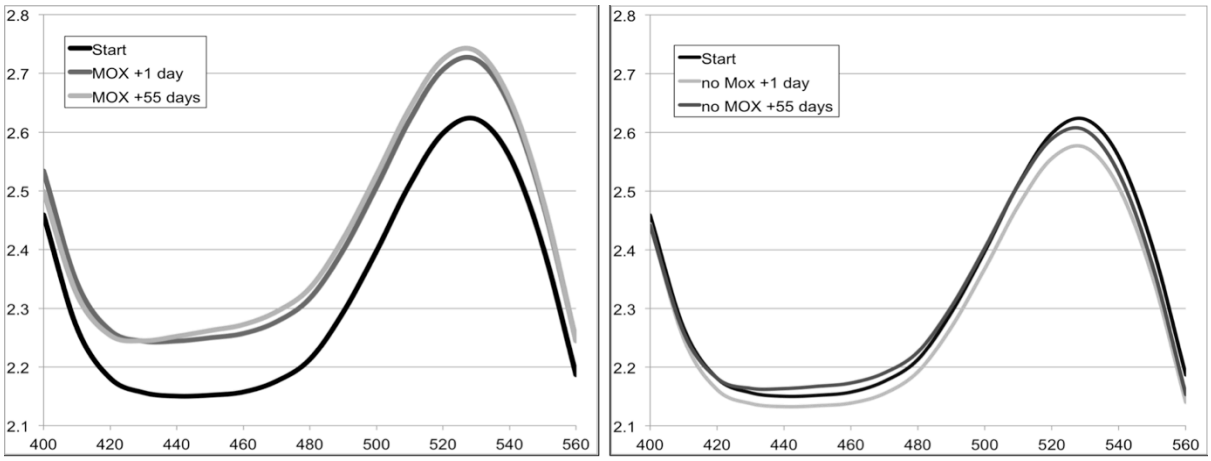

Fig. 18: Microoxygenation and shifting colour curves (left figure with MOX, right one without)

Accordingly, in the tanks subjected to microoxygenation, a significant gain in colour intensity and a slight nuance (hue) shift toward the red-orange spectrum was observed directly after the experiment and, in a more pronounced way, 55 days later (cf. Figs. 18, 19 and 20).

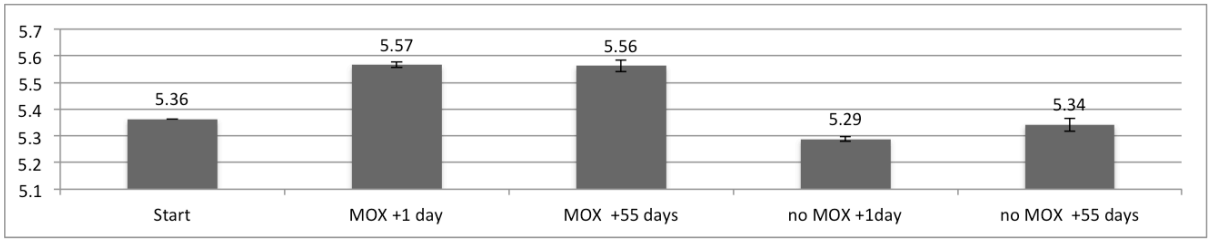

Fig. 19: Microoxygenation and wine colour intensity

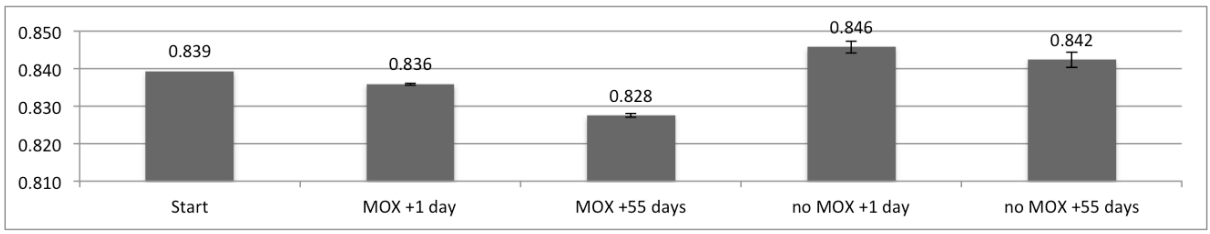

Fig. 20: Microoxygenation and wine hue

While an intensity increase in the tanks subjected to microoxygenation was observed across the entire colour spectrum, it was more pronounced in the wavelength bands corresponding to red (520 nm) and yellow (420 nm) (cf. Table 7 and Fig. 21). The more pronounced intensity gain in these bands is indicative for the stabilisation of red pigments and the formation of pyranoanthocyanins which have absorption peaks in the 520 nm and, to a lesser degree, in the 420 nm regions, leading to more orange hues (cf. Morata et al., 2006; Marquez et al., 2013). 55 days after the experiment, the intensity gains in the 520 nm region were even more pronounced. It is not clear, however, how much of the very significant vinylphenol reduction is responsible for this very slight shift in hue. A pertinent question (for future research) is how much vinylphenol reduction is needed to cause 1% of change in hue, when this is inputted to the formation of pyrano anthocyanins.

Table 7: Changes in wine colour intensity and nuance (hue)

	420 nm (yellow)	520 nm (red)	620 nm (brown)	Intensity	Hue
MOX +1 day	+ 3,73%	+ 4,17%	+ 2,26%	+ 3,78%	- 0,42%
MOX +55 days	+ 3,36%	+ 4,84%	- 0,01%	+ 3,71%	- 1,41%
no MOX +1 day	- 0,89%	- 1,64%	- 2,25%	- 1,40%	+ 0,77%
no MOX +55 days	+/- 0,00%	- 0,35%	- 2,04%	- 0,39%	+ 0,36%

Conversely, in the tanks not subjected to microoxygenation, a significant loss of colour intensity was observed, as well as a nuance shift towards the yellow spectrum. 55 days after the end of the experiment, these intensity losses and nuance shifts were partly reversed, yet without getting back to the levels measured at the start of the experiment.

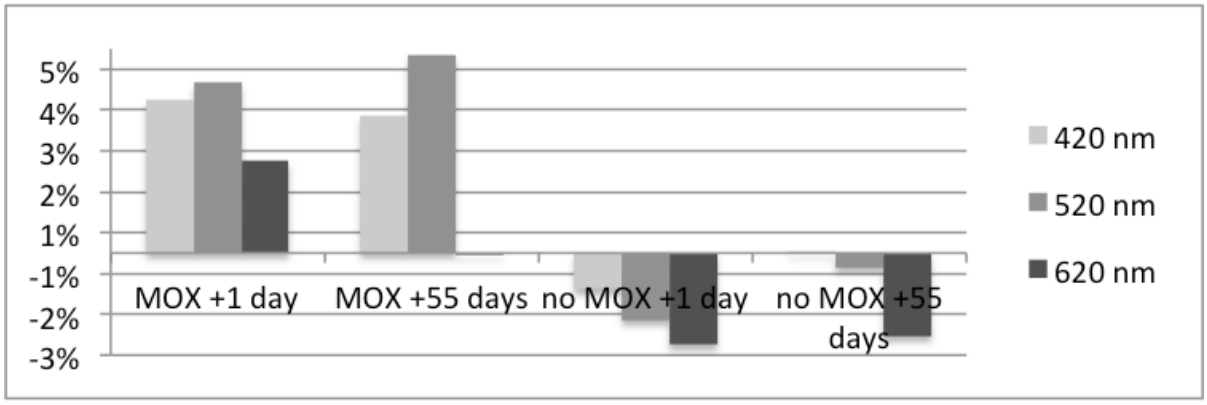

Fig. 21: Microoxygenation and wine colour shifts (in percentage)

4.6 Microoxygenation and measurable acetaldehyde and pH values

The acetaldehyde in the wine was relatively high already at the beginning of the experiment (49 mg/L) and did not significantly alter during the microoxygenation phase. Contrary to what would be expected (i.e. an increased formation of acetaldehyde through the oxidation of alcohol), the concentration of acetaldehyde in both groups of tanks, with and without microoxygenation, remained stable at a value of 49 mg/L. One explanation could be that the additional acetaldehyde produced in the tanks subjected to microoxygenation were directly used for the formation of bonds with anthocyanins and tannins, and hence broken up at the rate of its production.

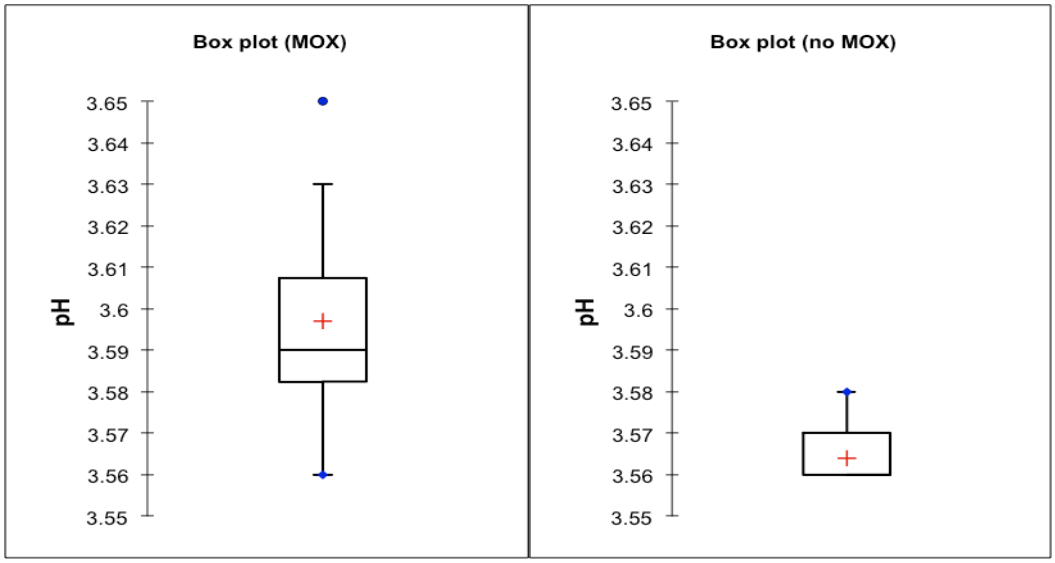

Fig. 22: Microoxygenation and pH (one day after the experiment)

The pH value in the tanks subjected to microoxygenation went very slightly up (by 0,04) while it remained stable in the untreated tank series (cf. Fig 22). This might be due to increased microbial – especially fungal and bacterial – activity in the presence of oxygen.

(As mentioned above, a number of fungi and bacteria were found in the wines at the end of the experiment.) A later test of artificially increasing the pH of the base wine by 0,04 and verifying changes in colour intensity and hue produced negative results. In this sense, the changes in colour described in the section above cannot directly be inputted to change in pH (which may have been caused by microbiological activity).

4.7 Sensorial test confirms reductive effect of microoxygenation on vinyl compounds

In a triangular test, a panel of 18 trained tasters (oenology students from the University of Geisenheim) found significant differences between wines that were, or that were not, subjected to microoxygenation only in the tanks spiked with vinylphenol and vinylguaiacol (cf. Table 8). Considering the significant reduction of these compounds affected by microoxygenation (reaching end values below reference sensorial thresholds), it comes to little surprise that these differences have here been detected. At the same time, tasters were unable to find a significant difference between pairs of wines that contained both vinyl and ethyl compounds, and in which the oxygenation had had a similar effect of vinyl compound reduction. A plausible explanation is that the stable concentration of ethyl compounds masked the reduction in vinyl compounds. This would be indicative of the aromatic dominance of ethyl compounds in the presence of both types of phenols.

Table 8: Triangular test of sensorial differences caused by microoxygenation

	MOX vs. no MOX	***Answers***	***Answers right***	***Significance***
1	Base wine	18	7	0,3915
2	Ethyl vinyl (16:4)	18	6	0,5878
3	Ethyl+vinyl (8:2)	18	7	0,3915
4	Ethyl (8:2)	18	9	0,1076
5	Vinyl (8:2)	18	**10**	**0,0433**

Once a significant difference among wines subjected or not to microoxygenation was found only in the series of wines spiked with vinylphenol and vinylguaiacol, the preference test that was included in the triangular test only provides significant results for this pair: a clear preference was given to the wine not subjected to microoxygenation. This may be related either to the remaining higher concentration of vinyl compounds (which tasters would appreciate in this case), or the production of other off-flavours in the course of microoxygenation. The first hypothesis seems more likely because had off-flavours been produced, they would very likely also be found in all other tanks subjected to microoxygenation and hence have led to significant sensorial differences, which were not

observed. The most likely explanation is that vinylphenol and vinylguaiacol in the concentrations added to the wine here positively enhanced the overall wine aroma.

4.8 Slightly bretty wines ranked top, intensively bretty wines ranked lowest

In a second series of trials, a total of 70 tasters participating in 4 separate panels (16 in Bordeaux, 23 and 18 in Geisenheim and 13 in Lisbon) were asked to rank from most-liked (rank 1) to least-liked (rank 5) the two series of wines (with and without microoxygenation). The wines were only smelled. The sum of ranks was calculated by multiplying the times each rank was selected by its value (i.e. 1, 2, 3, 4, 5). A very low sum of ranks (minimum 70 for 70 tasters) indicates a much-liked wine while a high sum of ranks (maximum 350 for 70 tasters) denotes a much-disliked wine.

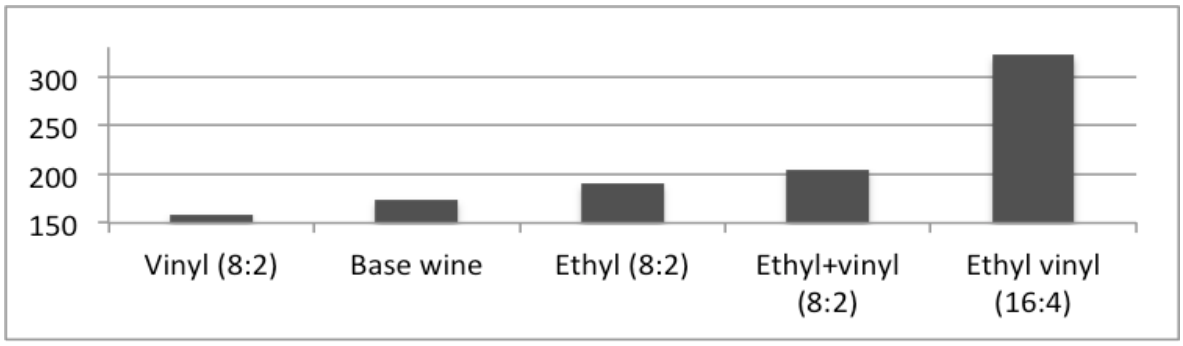

Fig. 23: Sum of ranks (wines with microoxygenation) for 70 tasters

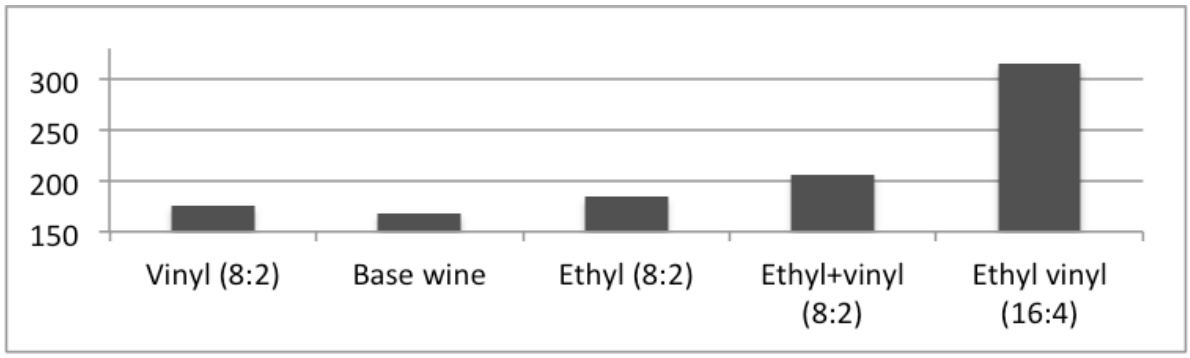

Fig. 24: Sum of ranks (wines without microoxygenation) for 70 tasters

By means of a Tukey range test, two significantly different groups of wines within the series of all samples was verified (for each respective taste panel): the wines spiked with the highest concentrations of ethyl and vinyl compounds were significantly and consistently ranked in last place (both in series with and without microoxygenation). While no statistically significant differences were observed in the ranking among the four other wines when looking at each taste panel separately, the overall result based on all tasters that participated in the ranking tests provided a significant outcome.

As a general rule, it was observed that the more the wines were spiked with additives the least the tasters liked them (cf. Fig. 23 and Fig. 24). The only exception to this rule, already observed in the previous section, was the observation that the presence of vinyl compounds in low concentrations seemed able to enhance the overall aromatic expression of the wines – or that their significant reduction via microoxygenation makes them undetectable (cf. Fig. 13 and Fig. 14 in the section on vinyl reduction, which indicate post-microoxygenation levels below the respective sensorial thresholds).

4.9 Fruity aromas are perceived as pleasant, phenolic aromas as unpleasant

The observations above are further confirmed by a descriptive test of each wine carried out by the same four taste panels. The spider net diagrams for the aroma and preference profiles of the wines perceived as least 'pleasant' and most 'pleasant' (the base wine without microoxygenation and the wine with the highest concentration of phenolic additives, respectively) indicate a clear distinction between fruity and savoury-animal-chemical aromas, the first considered as pleasant, the second mainly as unpleasant (cf. Fig. 25).

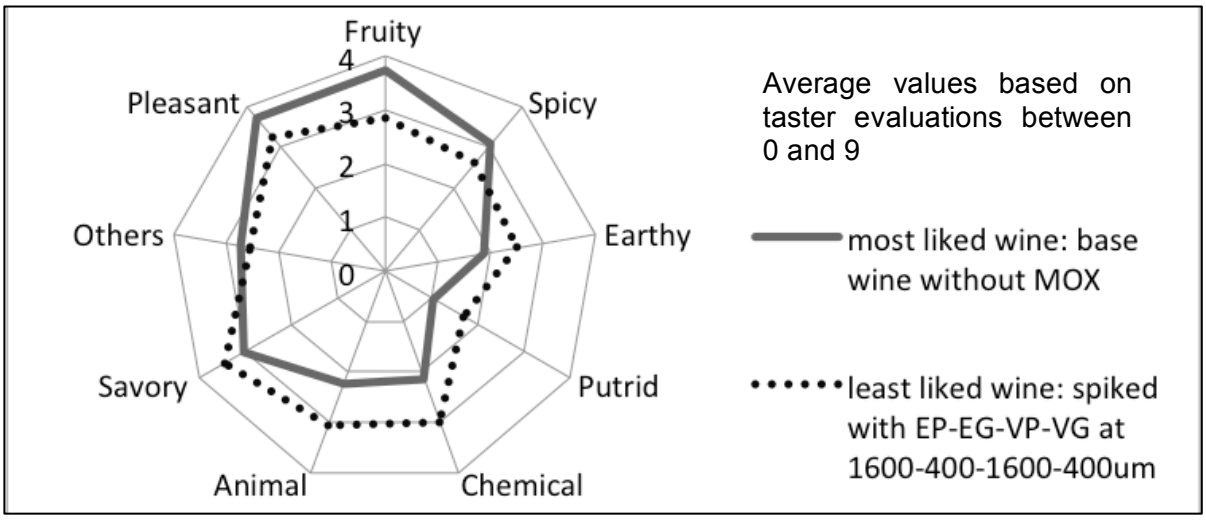

Fig. 25: Aroma profiles of most and least 'pleasant' wines

Among the 4 taste panels and all tasters as a whole, the covariance matrixes of aroma descriptors show a clear correlation between the descriptors 'pleasant' and 'fruity' (and to a lesser degree of 'spicy' and 'savoury') on the one hand, and the phenolic characters: 'animal', 'putrid', 'chemical', 'earthy' and, to a lesser degree, 'savoury' on the other (cf. Table 9 and Figs. 26, 26a-d). The descriptors 'pleasant' and 'fruity' are consistently negatively correlated to the phenolic descriptor group formed by 'animal', 'putrid' and 'chemical.

Table 9: Correlation matrix between aroma descriptors

	Fruity	Spicy	Earthy	Putrid	Chemical	Animal	Savory	Others	Pleasant
Fruity	1,000	**0,230**	-0,161	**-0,226**	-0,161	**-0,369**	0,014	**0,204**	**0,549**
Spicy		1,000	0,185	-0,005	-0,006	-0,017	**0,268**	**0,287**	**0,267**
Earthy			1,000	**0,330**	0,152	0,270	**0,232**	0,065	-0,123
Putrid				1,000	**0,254**	0,415	0,122	-0,042	-0,179
Chemical					1,000	0,208	0,149	0,042	-0,136
Animal						1,000	**0,362**	-0,106	**-0,308**
Savory							1,000	**0,214**	0,055
Others								1,000	0,208
Pleasant									1,000

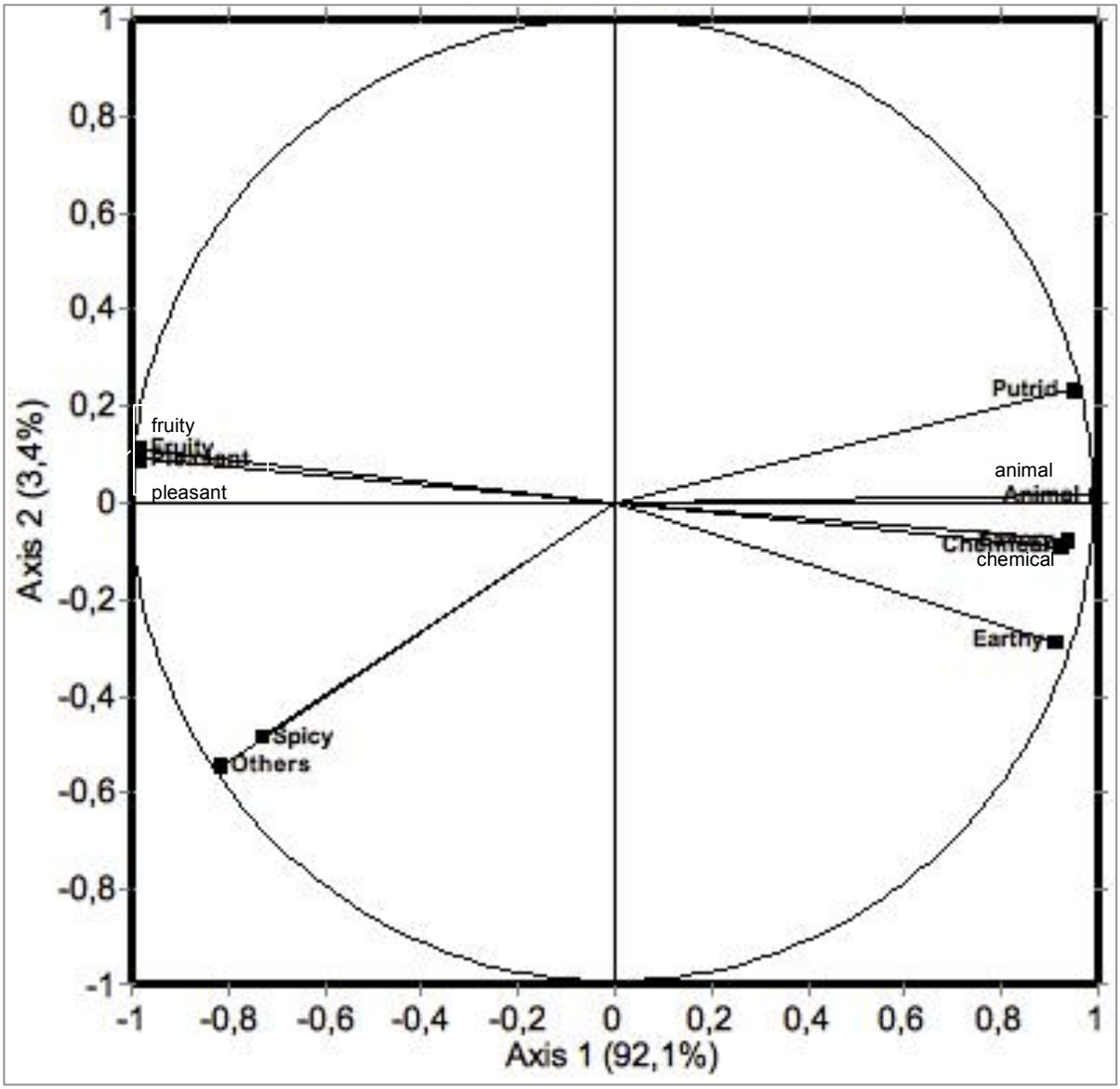

Fig. 26: PCA of aroma descriptors for all tasters

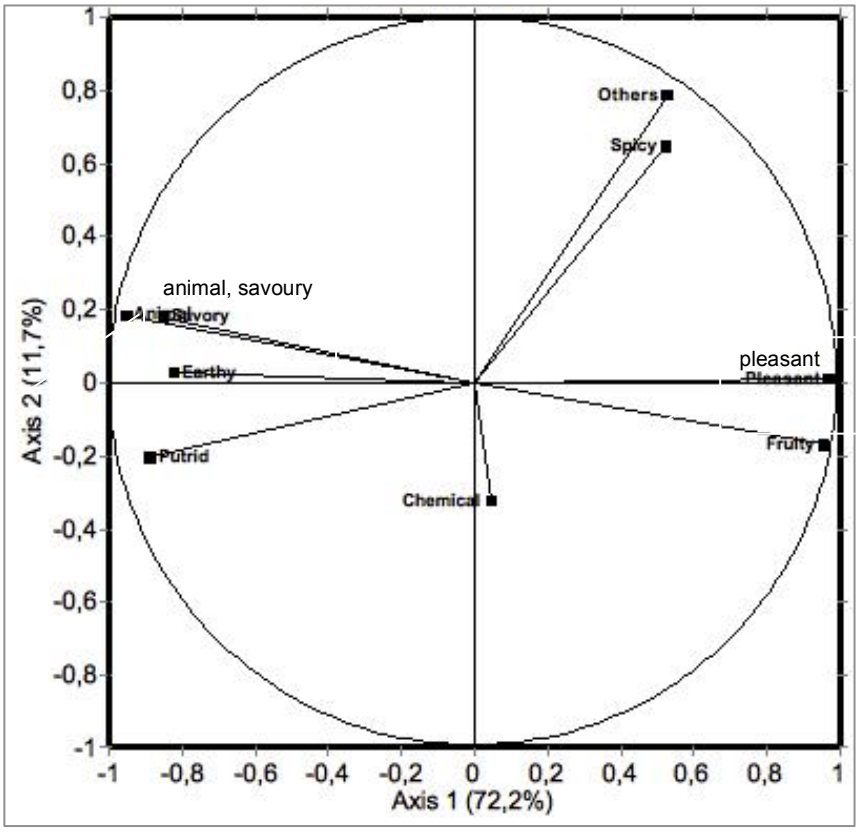

Fig. 26a: PCA of aroma descriptors for the Geisenheim MSc Enology taste panel

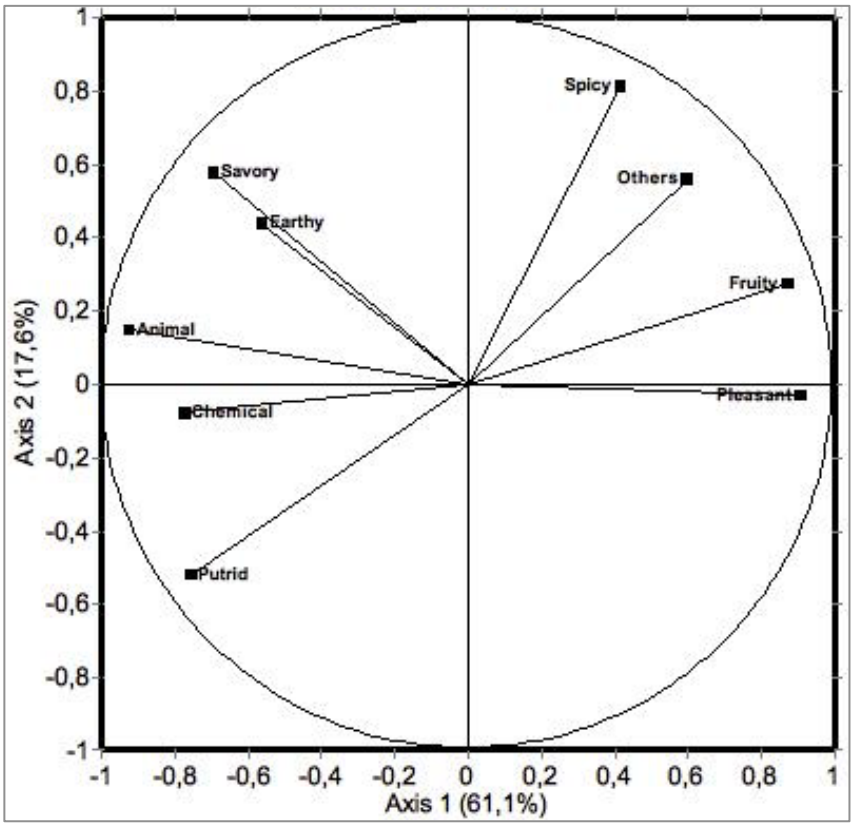

Fig. 26b: PCA of aroma descriptors for the Geisenheim MSc Vinifera-Vitis Vinum panel

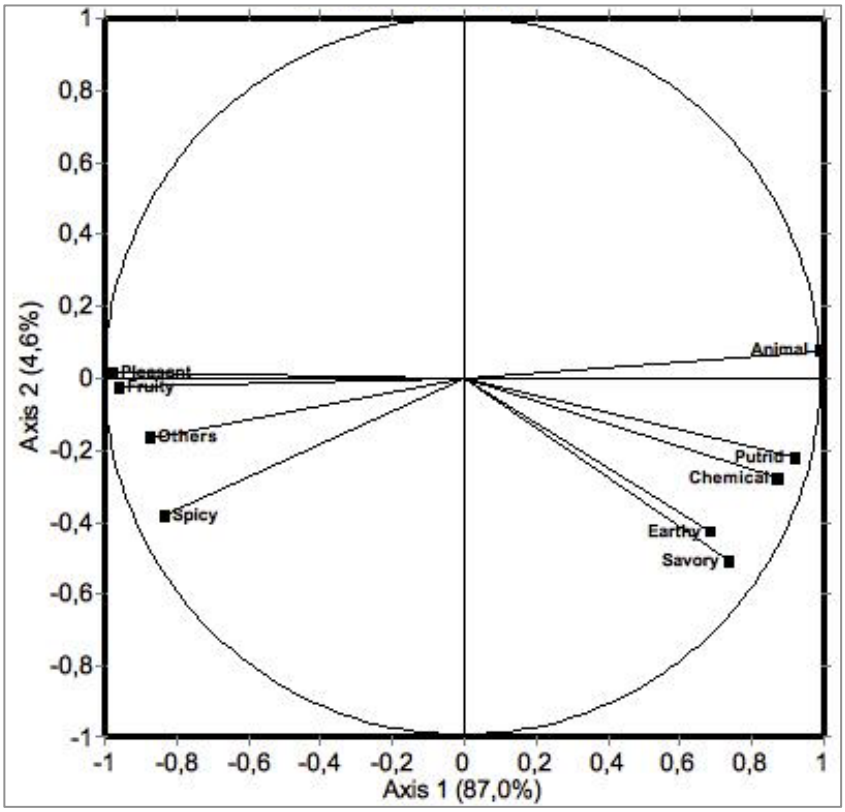

Fig. 26c: PCA of aroma descriptors for the Bordeaux DNO taste panel

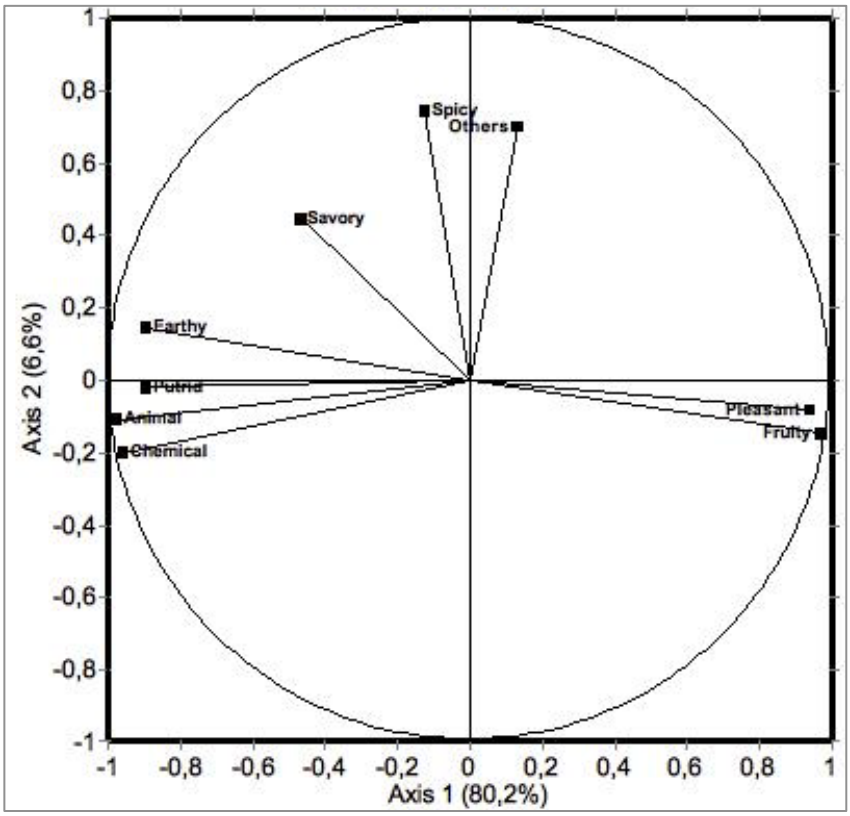

Fig. 26d: PCA of aroma descriptors for the Lisbon MSc Enology taste panel

Minor differences between taste panels concern the descriptors ‘spicy’, ‘savoury’ and ‘chemical’ – with no clear correlation patterns for ‘spicy’ and ‘savoury’ among the Lisbon panel, and no clear correlation for ‘chemical’ among the Geisenheim group. An explanation may be that these descriptors have, respectively, not been taught as part of the tasters’ training or are not generally understood within the cultural contexts of the tasters (cf. also last section of this chapter).

4.10 Microoxygenation has no measurable general effect on aroma or preference

In the winemaking world, it is sometimes assumed that microoxygenation leads to fruitier wines (cf. Smith, 2013). The research does not confirm this assumption. To the contrary it does not detect any specific general logic regarding the impact of microoxygenation upon a wine’s aroma profile (other than reducing ethylphenol precursors). These results must be dealt with care as the microoxygenation experiment in this case used a wine that was already a year old and took place after malolactic fermentation. The creation of fruity aroma by winemakers is usually related to the fermentation and post-fermentation phases.

To get to this finding, a Tukey range test was used to gather the wines in statistically significant groups defined by similar aroma profiles (cf. Table 10). Across the whole sample of wines, two major groups could thus be identified (emphasized in light grey and dark grey respectively in Table 10 – marked by A, B and C groups). These confirm the result of the previous triangular test, which singled out two wines – the ones spiked with the highest combined phenolic additives against the rest of all other wines.

Table 10: Wines regrouped by aroma descriptors (Tukey test at 5%)

	Fruity	Spicy	Earthy	Putrid	Chemical	Animal	Savory	Others	Pleasant
Base wine MOX	AB	A	AB	C	ABC	BC	AB	AB	A
Base wine no MOX	AB	A	AB	C	ABC	BC	B	AB	A
Vinyl (8:2) MOX	A	A	AB	C	BC	C	B	A	A
Vinyl (8:2) no MOX	AB	A	B	C	BC	BC	B	AB	A
Ethyl (8:2) MOX	A	A	AB	ABC	ABC	BC	B	AB	A
Ethyl (8:2) no MOX	AB	A	AB	BC	ABC	BC	AB	AB	A
Ethyl+vinyl (8:2) no MOX	AB	A	AB	C	C	BC	B	AB	A
Ethyl+vinyl (8:2) MOX	BC	A	AB	ABC	ABC	B	AB	AB	A
Ethyl vinyl (16:4) MOX	C	A	AB	AB	AB	A	A	AB	B
Ethyl vinyl (16:4) no MOX	C	A	A	A	A	A	AB	B	B
Comp. F	8,36	2,1	2,81	17,85	5,36	3,59	2,93	2,44	10,55
Proba.	***	*	**	***	***	***	**	*	***

* significant at 5 % ** significant at 1 % *** significant at 0,1 %

From this table, the less significant descriptors were then eliminated (those that did not allow to distinguish between different wines) and only the 3 most significant: 'fruity', 'animal' and 'pleasant' were kept. To calculate a value for what is called here the 'MOX factor', the difference in appreciation of a same wine without and with microoxygenation was computed (from the tasters' respective evaluations on a scale from 1 to 9). A negative MOX factor indicates that the wines scored lower after treatment with microoxygenation and, vice and versa, when this factor was positive, the wines scored higher after microoxygenation (cf. Table 11).

Table 11 shows that there is no general logic for the impact of microoxygenation upon the aroma and preference profiles of the wines used in this experiment. The results are fairly random. In some cases, the impact of microoxygenation is to make wines more animal, less fruity, and less pleasant. In other cases, it is the contrary: the wines become perceived as fruitier and more pleasant. These observations are independent from the types or quantities of phenolic additives. In this sense, within the framework of this experiment, the work shows that no detectable form of aromatic integration takes here place through microoxygenation (other than the above mentioned reduction of ethylphenol precursors).

Table 11: Microoxygenation has (here) no significant general impact on wine aroma profiles

	Fruity	Animal	Pleasant
Base wine	-0,48	0,75	-0,62
Vinyl (8:2)	0,31	-0,42	-0,13
Ethyl (8:2)	0,65	-0,22	0,41
Ethyl+vinyl (8:2)	-1,16	1,06	-0,5
Ethyl vinyl (16:4)	-0,06	-0,41	-0,03

4.11 Correlation between perceived aroma and actual phenolic concentration

To test how accurate the taste panel assessments were with regard to detecting actual chemical-physical differences among different wines, an excel worksheet was created with the data on the aroma and preference profiles of the different wines analysed by the taste panels and the actual phenolic concentrations in the wine at the end of the experiment (measured by gas chromatography). When computing this data, a good correlation between actual phenols and the respective perceived phenolic character (and appreciation) of each wine was verified.

The wines spiked with the highest concentrations of phenols yielded the highest positive correlations with typical phenolic character descriptors ('putrid', 'chemical', 'animal') and negative correlation with perceived fruitiness and pleasance (cf. Table 12). The two ethyl

compounds produced the clearest results. The vinyl compounds produced slightly less significant results; yet especially the correlation between vinylguaiacol and unpleasant animal aromas was significant.

Table 12: Correlation matrix comparing descriptor evaluations and actual phenolic concentrations

	Fruity	Spicy	Earthy	Putrid	Chemical	Animal	Savory	Others	Pleasant
EP	**-0,307**	-0,123	0,178	**0,274**	**0,207**	**0,478**	0,184	-0,165	**-0,367**
EG	**-0,303**	-0,128	0,179	**0,271**	0,199	**0,467**	0,181	-0,160	**-0,368**
VG	-0,188	-0,081	0,068	0,142	0,086	0,282	0,103	-0,088	**-0,205**
VP	**-0,230**	-0,096	0,100	0,184	0,125	**0,357**	0,126	-0,117	**-0,260**

These values are a statistical measure indicative for how much a certain quantity of phenols can overall (statistically) explain a certain perceived aroma descriptor based on all samples. It would make much sense to return to the lab and carry out a more systematic aroma perception test with model wine or uncontaminated young wine with no ethylphenol present, spiked with different levels of vinylguaiacol and vinylphenol. Also, the experiment did not allow the test of collinear effects due to the simultaneous presence of different phenols, which, in terms of sensorial analysis, is expected not to be additive (several phenolic characters at the same time) but transformative (a different aroma note due to the co-presence of different phenols), as will be shown in the next section.

4.12 Aroma intensification is not a linear phenomenon – stronger phenolic concentrations lead not to stronger, but to different aroma profiles

The preparatory phase of this experiment pointed at another dimension of the Brett paradox. During the preliminary trial carried out to detect aroma detection thresholds and preference sweet spots, it became noticeable that with increasing phenolic concentrations the tasters did not necessarily perceive an increasing intensity of a specific aroma note. Instead, many perceived that the wines acquired progressively changing aroma profiles.

For instance, when spiked with increasing concentrations of vinylphenol, the initial dominant aroma perceived in the wine was ‘savoury’, peaking at 600µg/L, then changing to ‘chemical’ when the concentration was further increased (cf. Fig. 27). Vinylguaiacol, in turns, initially presented an aromatic balance between ‘fruity’ and ‘animal’. Yet, with its increasing concentration, the dominant aroma of the wines became ‘savoury’ (cf. Fig 28). Ethylphenol, with increasing concentrations first seemed to mask the natural fruit aroma of the wine, still dominant at very low concentrations. With increasing concentrations, it then made the wine smell first savoury, then chemical, animal and again savoury (Fig. 29).

The paradox here seems to lie in the observation that the strongest concentration used in this experiment produces a wine that actually smells more pleasant than a wine with lower concentrations.

Further experiments were carried out with increasing concentrations of ethylguaiacol and of combinations of different vinyl and phenol compounds (Figs. 30-33). Here again, differences according to the respective concentrations, but also combinations were observed. These results should be considered with caution because the number of tasters was relatively low, and the answers effectively received for the later stages of the tasting get statistically less significant. This may be indicative for a fatigue effect caused by the physically challenging trial with aroma-intense wines.

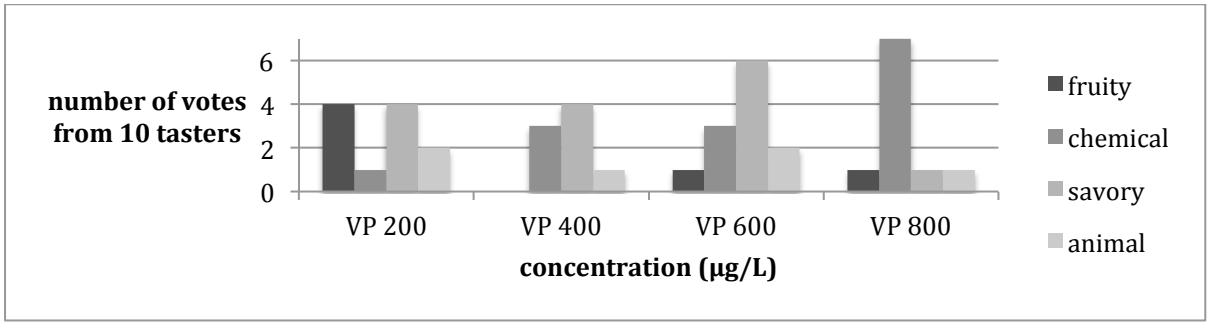

Fig. 27: Changing aroma descriptors with increasing vinylphenol concentration

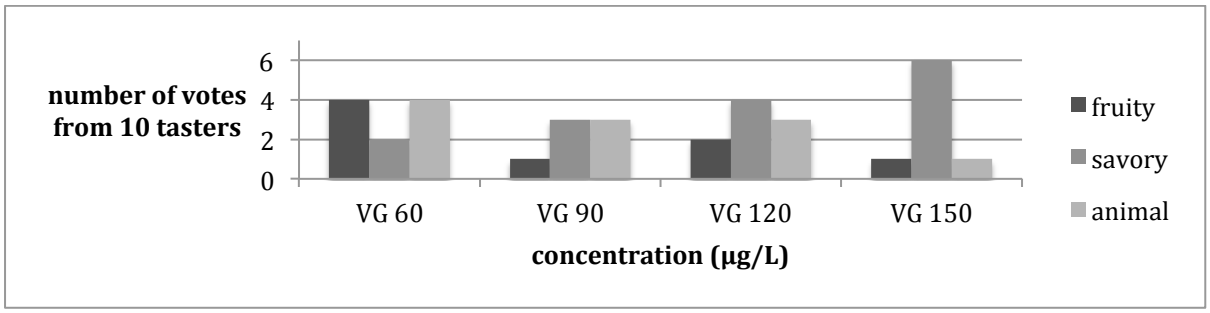

Fig. 28: Changing aroma descriptors with increasing vinylguaiacol concentration

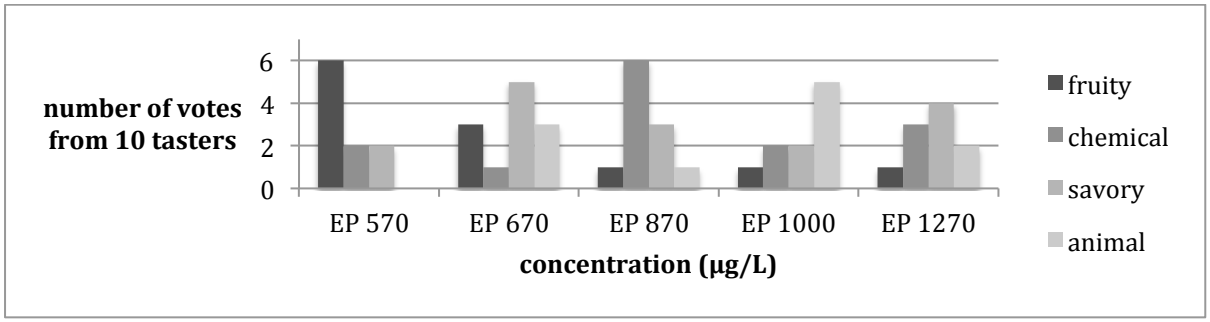

Fig. 29: Changing aroma descriptors with increasing ethylphenol concentration

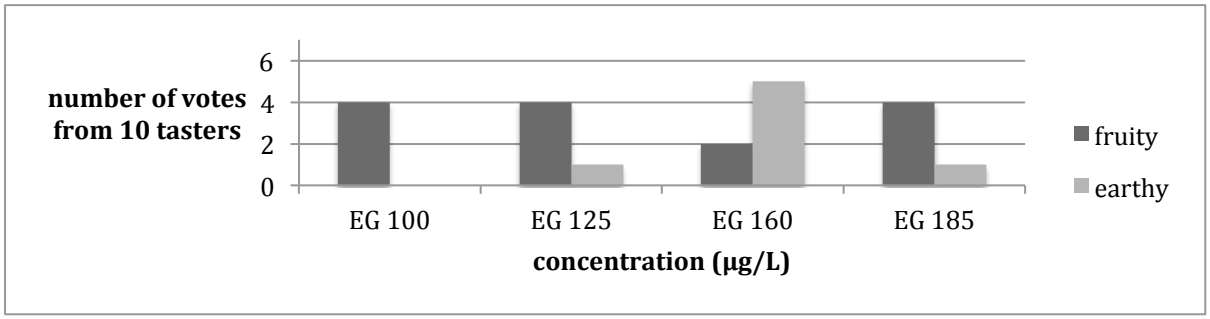

Fig. 30: Changing aroma descriptors with increasing ethylguaiacol concentration

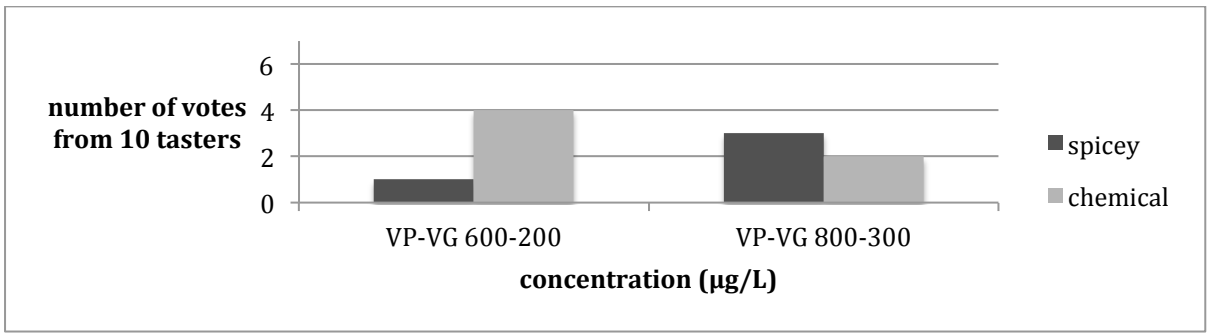

Fig. 31: Changing aroma descriptors with increasing combined vinylphenol-vinylguaiacol concentration

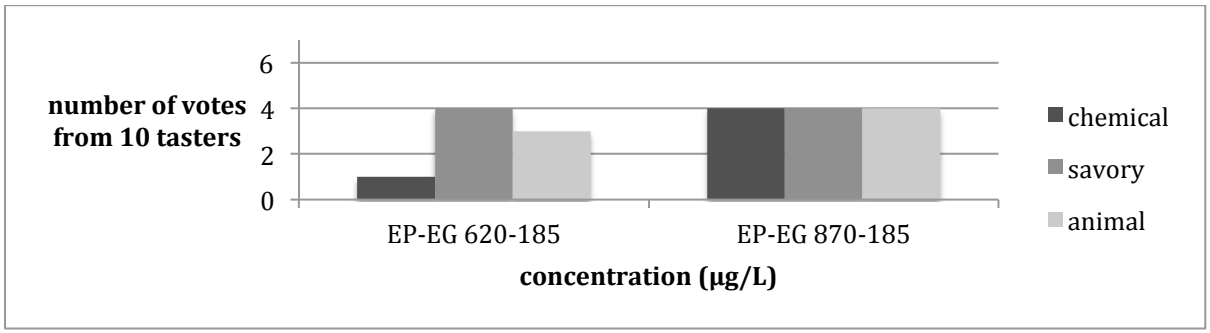

Fig. 32: Changing aroma descriptors with increasing ethylphenol-ethylguaiacol concentration

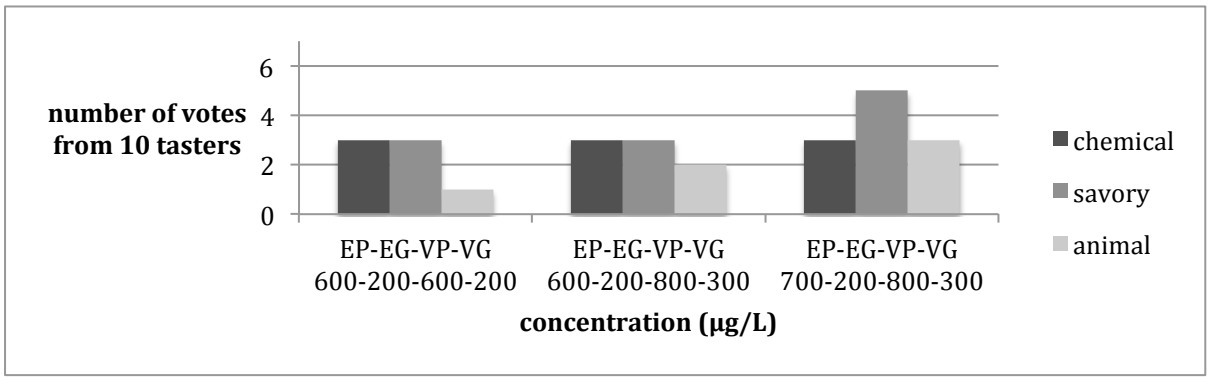

Fig. 33: Changing aroma descriptors with increasing concentration of ethyl-vinyl combination

As part of this trial, the tasters were also asked to attribute to each wine a number between 1 and 5 to evaluate how pleasant these were (1 denoting least pleasant, 5 most pleasant). The results of this second part of the study are in line with the previously described observations of aroma profile shifts with increasing phenolic concentrations.

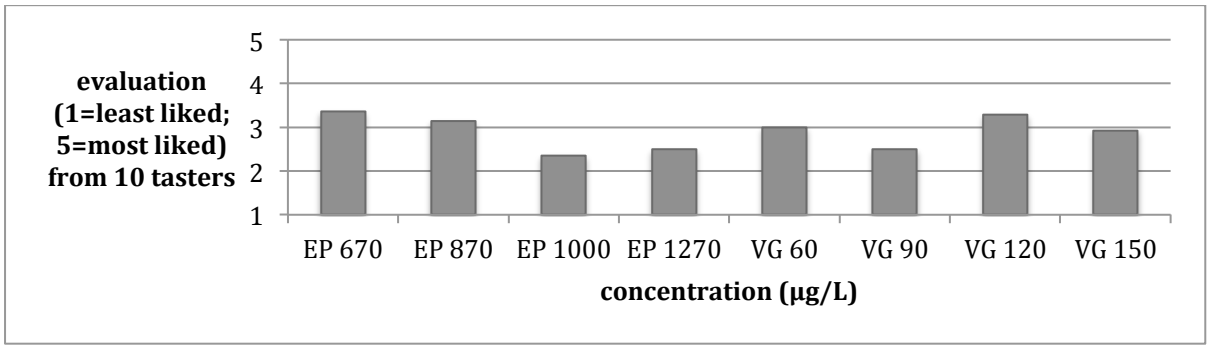

Fig. 34: Changing preferences with increasing ethylphenol and vinylguaiacol concentration

They confirm a research result manifest already earlier in this work: Increasing concentrations of Brett metabolites do not necessarily mean that the affected wines are perceived as less pleasant. However, all seems to depend on the types of phenols, their concentrations and their combinations with others. Ethylphenol, for example seems perceived as increasingly less pleasant when in higher concentrations, while a certain concentration of vinylguaiacol seems able to make a wine more pleasant (cf. Fig. 34), with more spicy aroma notes (Fig. 31). The preference sweet spot for vinylguaiacol among the tasters in this trial was at 120 µg.

The implication for winemaking is relatively limited. While, under certain conditions, vinylphenol could be great to have in wine as an aroma complexifyer, adding earthy or spicy notes, it is quite difficult to produce it in a controlled (and legal[4]) way and almost impossible to stabilise. For instance, it is not clear why this molecule would remain in a stable condition in finished white wines (as observed by Chatonnet et al. 1992). Certainly, most white wines do not undergo malolactic fermentation and are not aged in oak barrels, hence dramatically decreasing the risks of Brett infections. Saying that, as this experiment has demonstrated, vinyl molecules are highly reactive, with the risk not only of their transformation into ethyl derivatives (via the Brett metabolism or the action of *lactobacillus plantarum*), but also into a

[4] Within the OIV, it is not legal to add phenolic compounds to wine.

host of other possible non-controlled forms. This observation requires further research both on the stability of vinyl molecules (in white wines especially) and also their aromatic expression.

4.13 Social parameters (age, gender, country) explain some differences in taste descriptions and preferences

All tasters that participated in this research have been socialised and trained to wine in specific cultural and institutional contexts. This bears the question if the social parameters defining these contexts determine, at least partly, the way in which brettyness in wines is perceived. To test if the descriptive data produced by the different tasters was independent from their age, gender and taste group, an ANOVA was calculated testing the entire set of data against each respective social parameter. Some significant dependencies were observed. To further explore these, a series of Tukey (HSD) tests was carried out to verify the existence of statistical groups within each social parameter (i.e. over 30 years olds vs. under 30 year olds; male vs. female; Geisenheim panel vs. Vinifera panel vs. Bordeaux panel vs. Lisbon panel) who described and judged the wines differently.

Very minute differences can be revealed through the results, especially for different taste panels and to a lesser degree for gender. Overall, the Bordeaux panel evaluated the wines significantly differently from the three other panels and hence forms a statically distinct group within this data series. A closer look at the distinguishing elements of this group shows that the Bordeaux tasters attributed in general fewer values in each respective descriptive category and in a more pronounced way for the descriptors ‘chemical’, ‘putrid’ and ‘pleasant’. Different explanations are plausible: the Bordeaux tasters simply may not have liked the subjectively ‘unusual’ German red wine that they may have perceived as little pleasant. Alternatively, or complementarily, they may have been trained to a less generous general attitude towards attributing values. Conversely, the tasters of the Geisenheim panel generally attributed more values to all taste categories across the entire test, which may reflect a more generous attitude towards attributing values across their entire range. Such different general attitudes have nothing to do with wine tasting per se, but may reflect culturally different practices of assessing a same given reality (Black & Wiliam, 2005). Assessors (including of wine) trained in France may simply be more hesitant to attribute higher values than those trained in Germany.

Fig. 35 shows the differences between panels for the wines judged overall as the least pleasant (wine 2 with the highest concentration of phenolic additives), the most pleasant

(wine 6: base wine without microoxygenation) and the one with the most differentiated judgements among the national taste panels (wine 3 with a medium concentration of phenolic additives), much appreciated by the Lisbon panel while little liked by the Bordeaux panel.

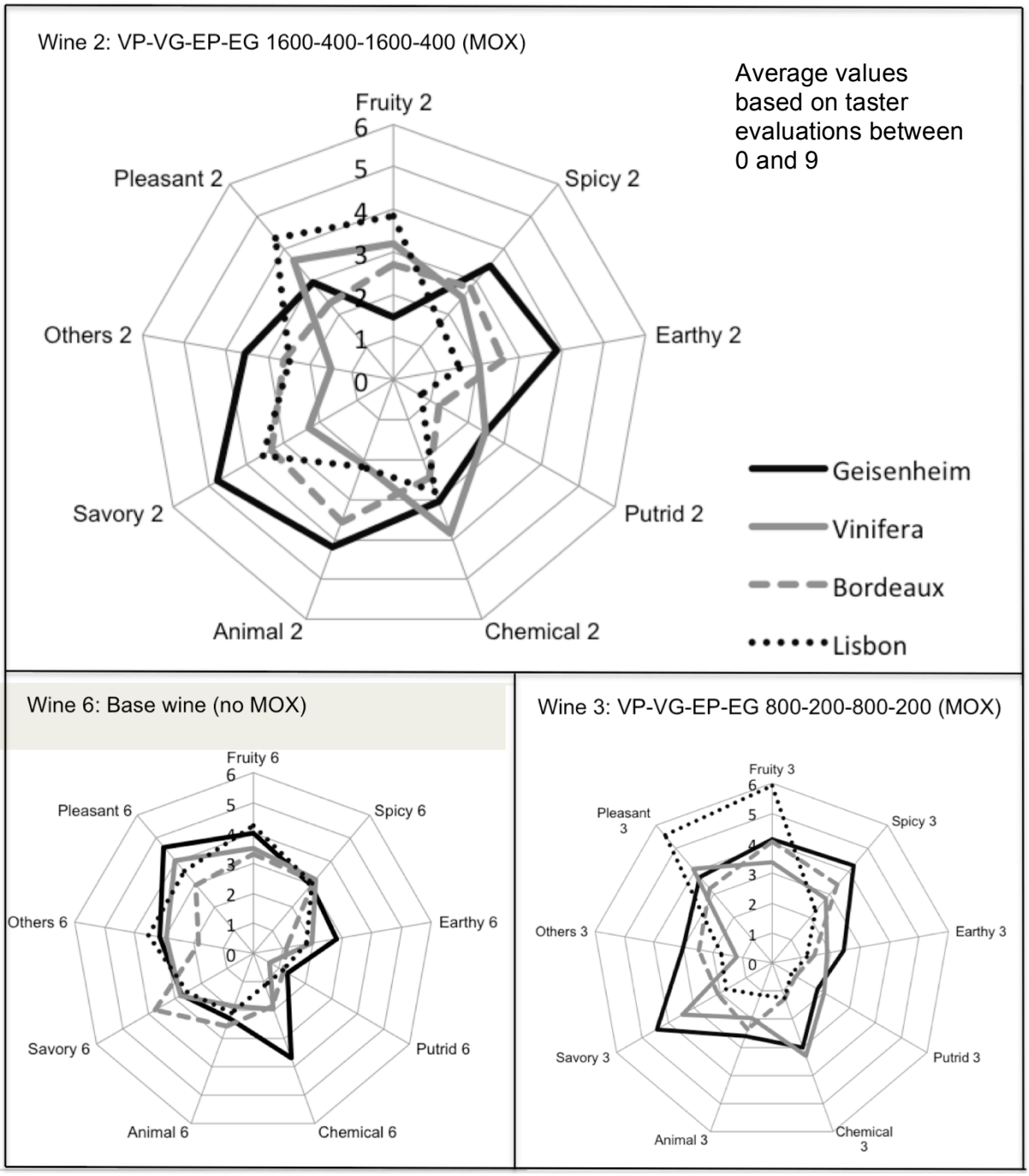

Fig. 35: Different aroma appreciations by national taste panels

Moreover, internal dynamics to the taste panels (which in all of the cases here are groups of students who know each other, have been trained together and hence form distinct epistemic communities) may bring about certain taste descriptor fashions. For instance,

among the tasters of the Bordeaux and Vinifera panels, ‘floral’ and ‘woody’ were frequent descriptors selected in the multiple choice part of the taste sheets, while these were almost totally ignored by the other tasters.

Also, as it was hinted at already earlier, some descriptors – such as ‘spicy’ for the Lisbon panel, or ‘chemical’ for the Geisenheim panel – did not produce any relevant results or correlations with other descriptors. In other words, they did not make sense as a reference for the respective tasters (whose distribution of values for these descriptors was statistically random). A plausible explanation is that these descriptors are outside both the cultural context and the institutional training of these respective panels.

Only very minute difference among wine descriptions were observed in terms of the gender of the tasters (which overall formed one statistical group). Gender-related descriptions were significantly different only in two aroma categories: ‘savoury’ and ‘others’, although the actual distribution of values as percentage of each gender groups’ total (Fig. 13) shows here nothing really outstanding. Maybe more of a curious nature (and open to discussion) is the observation that to describe the same series of wines, male tasters used more frequently the descriptor ‘cooked fruit’ while female tasters used more frequently the descriptor ‘dried fruit’.

Table 13: Gendered differences in aroma appreciation

fruity	*tropical fruit*	*citrus*	*dried fruit*	*cooked fruit*	*candy*	
male	14%	8%	14%	48%	16%	100%
female	7%	7%	39%	29%	17%	100%
savory	*Leather*	*smoked*	*whiskey*	*balsamic*	*nutty*	
male	56%	19%	5%	14%	6%	100%
female	48%	12%	12%	14%	14%	100%
others	*Floral*	*veggie*	*acetic*	*Dairy*	*woody*	
male	27%	11%	24%	6%	33%	100%
female	34%	8%	23%	7%	29%	100%

The social parameter of age (i.e. older than 30 years; younger than 30 years) did not produce any significant result. It must be noted that all tasters in the Bordeaux and Geisenheim panels were younger than 30, and that the Vinifera group concentrated the highest number of older tasters. However, it would make sense to verify age-related taste assessments and preferences through further research.

Conclusions

The aim of this work was to explore the potential and goodness of the notion of aromatic integration of Brett metabolites through microoxygenation. Through an experimental research set-up using micro-filtered tannin-rich red wine spiked with various concentrations of phenols and microoxygenation technology adapted to small tanks, the work produced a number of interesting, sometimes unexpected results.

The main result was to reject the hypothesis of aromatic integration of the Brett metabolite ethylphenol through microoxygenation. In the research set-up, the concentrations of this phenol remained stable throughout the experiment. Also, the taste panels did not detect significant differences between ethylphenol-spiked wines treated, or not, with microoxygenation. At the same time, the alternative hypothesis assuming that microoxygenation helps to reduce vinyl compounds, as the intermediary metabolite of the Brett metabolism, has been clearly confirmed. In the presence of oxygen, this reduction saw rates of up to 80% within a short ten-day period during which microoxygenation was applied. As earlier suggested by Morata et al. (2007), it may therefore provide a pertinent method to control the production of Brett aromas (through the reduction of ethylphenol precursors). A follow-on experiment with model wine further showed that the addition of oenological tannins and higher temperatures equally contribute to the reduction of vinylphenol, without producing additional ethylphenol.

If one talks about aromatic integration, as does the American winemaker Clark Smith (2013), one may need to re-think such a notion in terms of what is plausible in terms of organic chemistry. Ethylphenol constitutes a relatively stable non-polar molecule that is very difficult to condense or bind to other molecules. This is precisely the reason why it is technically so difficult to treat a wine that had been contaminated with Brett and that contains high levels of ethylphenol. Vinylphenol (and vinylguaiacol and vinylcatechol), conversely is a polar molecule that can more easily be bound to other molecules or be enzymatically transformed, as in the Brett metabolism. In the absence of microbiological activity (ensured through the pre-experiment micro filtering of the wine), aromatic integration hence was caused indirectly, through the reduction of vinyl compounds – and not through the integration of ethylphenol into the tannic structure of the wine, as suggested by Smith.

The experiment included a series of sensorial analysis panels to verify if aromatic changes could be detected in the wines treated with microoxygenation. In a triangular test, the tasters could detect no direct aromatic impact whatsoever, except form the before mentioned changes due to the direct reduction of vinylphenol. In this sense, the wine did not become fruitier or aromatically better integrated, as many winemakers and equipment providers claim as the result of microoxygenation. However, these observations should be treated with caution. The set-up of the experiment was possibly too short, and the wine also possibly already too old (it had already spend 12 months in a steel tank), to produce here properly significant results. In practice, microoxygenation is not applied to a finished wine but during the winemaking process, either during or after fermentation, or during aging.

The photo-spectrometric analysis of the wines showed that microoxygenation provoked a significant gain in colour intensity and a slight shift towards red-orange colour hues. The same observation could not be verified in the wines not subjected to microoxygenation, where the colour intensity actually slightly decreased. Several hypotheses are plausible to explain this phenomenon:

(1) In the presence of acetaldehyde as a bridging molecule (produced through the oxidation of ethanol), anthocyanins may have been condensed with the oenological tannins that had been added to the wine at the beginning of the experiment. However, while this hypothesis has been repeatedly verified in previous works by other authors (Chatonnet et al., 1992), it could not be directly reconfirmed here because the concentrations of acetaldehyde were relatively high in both series of wines already before and throughout the experiment (at 49 mg/L respectively), which should have been largely sufficient to allow such a condensation in equal proportions in both series of wines without any further oxygen addition.

(2) In the presence of oxygen, anthocyanins may have been condensed to the tannins directly, without the bridging molecule of acetaldehyde (cf. Fig. 36).

(3) In the presence of oxygen, anthocyanins may have been condensed through reactions with vinylphenol, pyruvic acid and/or acetaldehyde forming pyranoanthocyanins (cf. Fig. 4), as earlier observed and reported by Morata et al. (2006, 2007, 2007; cf. also Marquez et al., 2013).

(4) Additionally, anthocyanins previously bound to SO_2 and colourless, may have become free and available, and been condensed as part of any of the processes outlined in points (1) to (3) above, hence adding to the overall colour intensity of the wine. This hypothesis is

plausible because of the observed very low level of free SO_2 and relatively significant level of bound SO_2 (11 mg/L and 109 mg/L, respectively).

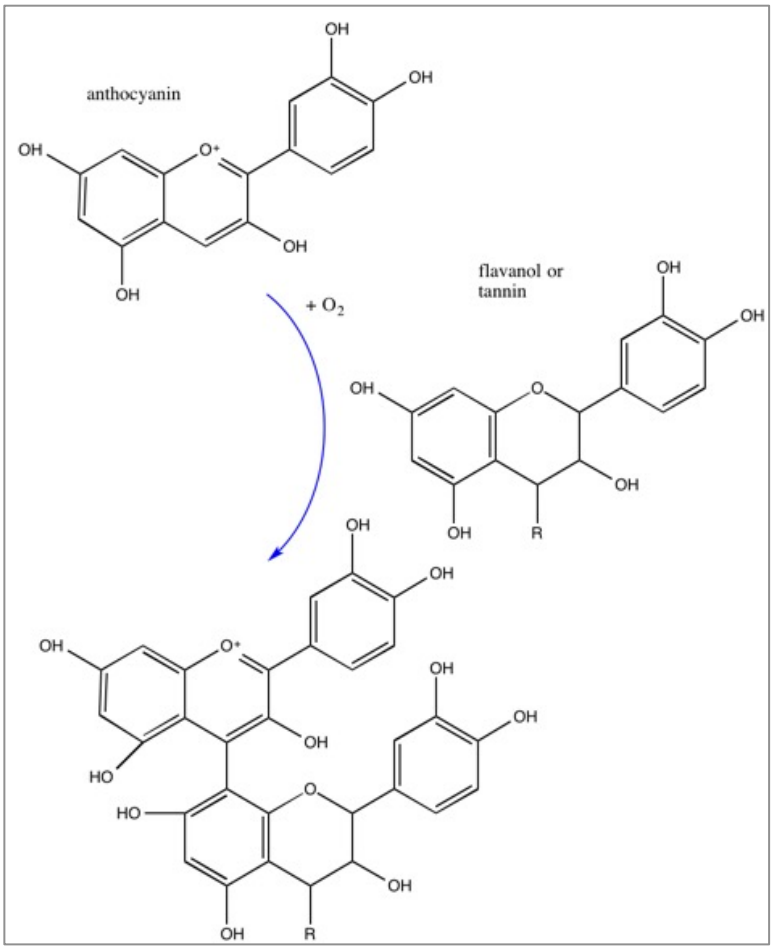

Fig. 36: Tannin-anthocyanin condensation in the presence of oxygen

A series of preference ranking tests with panels of trained tasters from the universities of Geisenheim (Germany), Bordeaux (France), Lisbon (Portugal) and Changins[5] (Switzerland) asserted the previously made observation that strongly bretty wines (spiked with high concentrations of ethyl and vinyl compounds) are clearly disapproved and discarded from a consumer-aesthetic point of view. However, the same taste panels showed that slightly bretty wines can score quite high in the preference rankings, especially when certain combinations of vinyl and ethyl compounds are present. This confirms an earlier observation

[5] Due to logistic and organisational problems independent from the University of Changins, the taste results from that panel only reached the author after the writing of this work. They do however confirm the results made in the other tastings.

on the paradox of bretty wines made by Eder (2010), showing a strong correlation between the brettyness of certain red wines and their esteem by international wine critics. The choice of the type of panellists – here all advanced oenology students trained to a pure-fruit aroma winemaking paradigm – hence seems crucial. It may make sense, therefore, to repeat the kind of taste panels carried out in this research with different types of panellists, for example wine critics, wine consumers or sommeliers.

The issue of subjective aromatic appreciation of wine was another interesting dimension to the question of aromatic integration explored in this research. To prepare the experiment, a preparatory sensorial analysis panel had been carried out to determine sensorial detection thresholds and preference sweet spots for different phenols. As an unexpected by-product, this panel showed that with increasing concentrations of phenolic additives, the wines were not perceived as progressively more aroma-intense (as was initially expected), but that they gradually changed their aroma profile entirely. In other words, a wine spiked with a low concentration of vinylguaiacol or ethylphenol had an aroma profile radically different from that of the same wine spiked with a higher concentration of the same additive. In terms of observed preference sweet spots, it was not always clear which wine was perceived as 'better' because, within the limit of the research set-up, different tasters perceived different amounts of phenolic characters as differently pleasant. Also, within the standardised normative framework of sensorial analysis taught as part of different university curricula in oenology, different tasters used different descriptors to characterize the same wines. It may make sense to follow up on these observations and carry out more focused research on the relation between different phenolic concentrations in a wine and the both aesthetic and semantic appreciation of its phenolic aroma profile.

The observation on the social variability of taste preferences and wine characterizations led to a second series of sensorial tests in which the differentiated responses by tasters of different gender, age and institutional affiliation (Bordeaux, Vinfera Euromaster, Geisenheim, Lisbon) were tested against each other. The results were eventually not very conclusive, other then showing that the different independent sample groups form an epistemic community producing largely converging responses with regard to wine aroma assessments and preferences. Mind differences were shown, mainly linked to a more or less consistent training of the students (the Bordeaux students produced the most homogenous, the Vinifera students the least homogenous descriptions of the wines).

Also some unexpected and curious observations could be made. For instance, when describing the same wines, the men among the sample used the descriptor 'cooked fruit' far

more often than the women, who used the descriptor ‘dried fruit’ with more frequency. Also, certain descriptors that are part of the aroma reference framework used in sensorial analysis seemed little understood by some national taste panels. The German tasters seemed unable to attribute a clear meaning to the descriptor ‘chemical’, and the Portuguese seemed unable to make sense, in a consistent way, of the descriptor ‘spicy’, which may be related, respectively, to different foci in the institutional training or even different national aroma cultures at large. It may make sense to follow up on these observations as well and carry out more focused research on such national (or regional, ethnic, class, age or gender) taste cultures.

The results of this research have a number of implications for oenological practice. At the currents state of knowledge and technological know-how, it seems rather difficult to refine bretty wine characters in a controlled way. The academic literature points toward the possible future identification and isolation of noble Brett strains that can positively complexify a wine’s aroma profile and add to its value. These could be developed into oenological products and be inoculated before or after the alcoholic fermentation, possibly in an isolated smaller tank that could later be stabilised and back-blended (to minimize risk of uncontrolled Brett contaminations).

Conversely, adopting a more ‘postmodern’ approach, as suggested by Smith, Brett yeasts which are marked by a very rich, globally distributed strain biodiversity could be reconsidered as part of terroir and the wine-making process. This would obviously mean to change winemaking protocols and accept unexpected outcomes – reflecting precisely what is meant by terroir – and also a certain randomness, which from an anthropological perspective translates nothing else than the age-old association of divine forces as part of the production process and of the magic and allure of the end product: wine.

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